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**LIVER: STRUCTURE
AND FUNCTION**



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Frontispiece. Pioneers in the study of hepatic structure and function. *a.* Francis Glisson (1597-1677) of London described the capsule of the liver and the portal circulation. *b.* Marcello Malpighi (1628-1694) of Bologna described the microscopic appearance of the liver. *c.* René Théophile Hyacinthe Laennec (1781-1826) of Paris coined the term *cirrhosis*. *d.* Karl von Rokitansky (1804-1878) of Vienna described acute yellow atrophy. *e.* Claude Bernard (1813-1878) of Paris demonstrated the endocrine role of the liver. *f.* A. A. Hijmans van den Bergh (1869-1943) of Utrecht initiated the study of bile pigment metabolism. *g.* Ludwig Aschoff (1866-1942) of Freiburg described the reticuloendothelial system. *h.* Hans Eppinger (1879-1946) of Vienna systemized liver diseases by clinical-pathologic correlations. (Figures *a* to *g* from the portrait collection of the Northwestern University Medical School Library.)

LIVER: STRUCTURE AND FUNCTION

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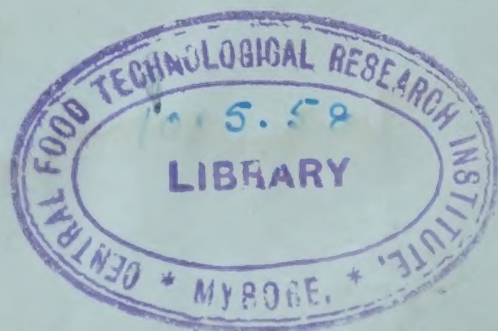
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The Blakiston Division

McGraw-Hill Book Company, Inc.

New York Toronto London 1957



LIVER: STRUCTURE AND FUNCTION

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Library of Congress Catalog Card Number: 56-7566

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Liver Structure

*To our patient wives for their enthusiastic help and understanding and to
our children, asking forgiveness for the many long hours away from them*



PREFACE

A disease is understood only if the functional derangement can be clearly associated with the morphologic changes observed during life or on the autopsy table. Introduced by the founders of present-day medicine, this is the recognized credo of students of human illness. Despite the efforts of clinicians and pathologists, the task of correlating the clinical manifestations of liver diseases, the functional derangements as recognized in the laboratory, and the morphologic alterations remains unfinished.

The basic functions of some organs, such as the kidney, are well established. Clinical tests have been devised to measure them, the results of which can be well correlated with the anatomic lesions and clinical findings. The basic functions of the liver are not so well understood, and few tests are available to measure them. Furthermore, the functional alterations resulting from many anatomic changes are still unexplained. Consequently, a classification of basic hepatic disorders can be made only on a descriptive or etiologic basis.

Each patient with liver disease presents anew the problem of correlation between disturbed function and structure. Our present-day fragmentary knowledge has to be applied to each case individually—appraisal cannot rest upon well-established associations. The practicing physician will need what little correlated knowledge is available for the diagnosis and management of hepatic diseases. This book attempts to assemble current concepts of hepatic structure and function for the benefit of the physician. Emphasis is placed upon the basis, the method, and the practical application of each of the so-called “liver function tests.” The diseases are classified on the basis of experience with liver biopsies. Finally, the central position of the liver, anatomically as well as metabolically, suggests a discussion of its relation to other organs as well as its response to external stresses.

Despite the title, which may imply an academic scope, only those aspects of hepatic physiology and pathology which have practical significance will be considered. Other aspects will be excluded, possibly because of lack of foresight. Since so many physiologic processes are represented in the liver, such restraint is necessary to avoid an encyclopedic treatise on general physiology and biochemistry. It also appears unwise to present the clinical features or therapy of a large number of diseases with the excuse that the liver may also be involved. In general, clinical features which are obvious and well described in standard textbooks of medicine are omitted in order to focus interest on the crucial point of correlation of hepatic function and structure.

The chapter divisions follow the modern trend of emphasizing pathophysiologic phenomena rather than diseases. The disease entities are therefore considered as examples of these phenomena, several of which may be involved in a single disease. Moreover, the normal function and structure, being more soundly established, are discussed in considerable detail, whereas the pathologic function and structure are considered in a broader sense. Thus references to basic phenomena do not complicate the descriptions of disease processes—the interested reader is referred to the preceding chapters.

The proper place for the discussion of the so-called liver function tests was not easily decided upon. If the tests were based on a clear understanding of hepatic physiology,

the discussion concerning them would logically follow the chapter on normal function. At present, however, the principles of the hepatic function tests touch only the fringes of physiology. The approach today is still elementary and subject to change. The tests, in general, reflect pathologic phenomena without identifying their underlying cause. It therefore seems appropriate to discuss the tests as a basis for diagnosis, with the object of establishing a usable system, rather than as an appendix to the section on physiology.

This book has been made possible by the generous support, helpful criticisms, and personal efforts of many persons. Dr. Karl A. Meyer, the medical superintendent of the Cook County institutions and president of the Hektoen Institute for Medical Research of the Cook County Hospital, was a constant source of encouragement and placed at our disposal the facilities of the Cook County Hospital with its laboratories and affiliated institutions. Heartfelt thanks are due to Dr. Samuel J. Hoffman, the executive director of the Hektoen Institute, who not only offered invaluable friendly advice but also generously assisted in overcoming innumerable difficulties throughout the writing of this book.

We are deeply obliged to many of our colleagues who read the manuscript or parts of it and offered very helpful suggestions and corrections; Drs. Sheldon S. Waldstein, Richard B. Terry, Jesus de la Huerga, Geza G. Kopstein, Alvin Dubin, Irving A. Friedman, and Isidore Snapper deserve special mention. We wish to express our appreciation to the many younger members of the clinical staff of the hospital who uncovered the clinical material and performed many of the biopsies, and to the staff of the department of pathology, whose heavy workload was increased by the collection of material for this book. Much help was provided by Dr. Paul B. Szanto, not only in collecting material but also in offering valuable criticism of the manuscript, which he carefully read. Further stimulation came from the many coworkers with varied fields of interest at the Hektoen Institute who joined in the endeavor to correlate hepatic function and structure. The experiences and observations of Drs. Elias, Farber, Franklin, de la Huerga, Koch-Weser, Kozoll, Meyer, Steigmann, Szanto, Terry, Volk, and Waldstein, as well as the biochemical analyses of Dubin and Dyniewicz, deserve special recognition. Our staff photographer, Harold L. Miller, was untiring and enthusiastic in his efforts, extending through many years, to provide the pictures used. Angela Bartenbach spent much time in her skillful execution of many of the drawings.

The authors wish to thank those who permitted the reproduction of illustrations published elsewhere, particularly Drs. Hans Elias and Frank Netter. We are indebted to Elizabeth F. Carr, Anna A. Carr, and Georgia Price of the Northwestern University Medical Library for the careful check they made of the references. Thanks are due to the several secretaries who faithfully did the necessary typing.

We feel a sense of obligation to the Josiah Macy, Jr. Foundation and the members and guests of the Conference on Liver Injury for the inspiration they provided one of us who was privileged to attend the Conference.

Many of the original conclusions in this book are based on the study of specimens which friends and colleagues all over the world have sent to our laboratory for study. We use this opportunity to acknowledge our gratitude to them.

Much of the original work upon which this book is based was carried out with the aid of grants generously given by the Jerome D. Solomon Memorial Research Foundation and also by the National Institutes of Health of the United States Public Health Service and the Committee on Scientific Research of the American Medical Association.

*Hans Popper
Fenton Schaffner*

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Introduction

The shape of the liver aroused interest several thousand years before the birth of Christ. A Babylonian model of the liver kept in the British Museum shows segmentation which matches well the results of modern injection techniques (Fig. 1). The shape of the liver in sacrificial animals was thought to bear omen about the future, and the haruspex, or diviner inspecting the viscera of such animals, was believed to learn much from the size of the right or left lobe. The shape of the liver was consulted before the warrior went to

the Theban War (Fig. 2). An Etruscan liver model found in central Italy shows a similar, although less elaborate, shape. Little curiosity existed to explore the relation of this shape to the health of the animal itself, and a similar approach was made to human necropsy specimens. Autopsies were performed by the Egyptians in which the heart weight was recorded but relatively little concern was exhibited for correlation between the shape of the organs and their function. All through the older necropsy studies, including the older



FIG. 1 Clay model of sheep liver made by Babylonian priests, 2000 B.C., preserved in the British Museum, London. (From the portrait collection of Northwestern University Medical School Library.)



FIG. 2 Diviner (haruspex) showing the liver of a sacrificial animal to Adrastus, a Greek warrior departing for the Theban War. Drawn on a Greek vase. (From the portrait collection of Northwestern University Medical School Library.)

German or Italian ones, the shapes of the altered organs were in the foreground of interest of the physicians.

As biological knowledge improved, the study of structure became more elaborate. Analytical data obtained by more and more exact measurements became the goal of the different biologic disciplines such as anatomy, physiology, biochemistry, and biophysics. The cellular pathology of Rudolf Virchow opened the finer structural elements of the body to investigation. This analysis of altered microscopic and even submicroscopic structure for its own purpose is best exemplified in the fundamentalist approach, especially found in the German school of pathology. Ricker, as the most articulate spokesman of this line of thinking, considered pathology as a natural science in its own right, its aim being to study the structural changes of disease for its own purpose, with little more concern for the clinical implications than the Babylonian or Greek haruspices showed when they studied the shape of the liver to predict the future.

A synthetic approach to pathology, the attempt to correlate structure and function or, in other

words, to relate the findings of the autopsy table with clinical observation, came into flower only in the amphitheatres of the later classic Italian schools. Two men contributed much to this development in pathology. Anthony Benivieni, whose *De abditis nonnullis ac mirandis morborum et sanationum causis* (About Many Unusual and Miraculous Causes of Diseases and Cures) appeared in 1507, was a forerunner of Giovanni Morgagni, whose *De sedibus et causis morborum per anatomen indagatis* (About the Sites and Causes of Disease Investigated by Anatomy) appeared in 1761. Many physicians from all over the Western world came to the Italian amphitheatres, and in Bologna autopsies were performed under the inscription which we today proudly exhibit in the amphitheater of the Department of Pathology of Cook County Hospital: *Hic locus est ubi mors gaudet succurrere vitae* (This is the place where death enjoys helping life). The approach of having structure and function correlated by an investigator who was both clinician and pathologist is best exemplified by the great leaders of the English and French schools of the later eighteenth and nineteenth centuries; men such as Hodgkin

Bright, Laennec, and others, who went from the autopsy tables into the wards. This approach was expressed in the writings of the Austrian Karl Rokitansky. He did not neglect to make most careful anatomical observations, yet he also emphasized the correlation with the basic sciences, such as chemistry, for understanding pathologic processes, not for obtaining analytical data for its own sake. In contrast to the fundamentalist approach, pathology became a correlative or integrative science, differing from the analytical biologic sciences by having no technique but an approach of its own, using all morphologic, chemical, and other data for correlation with the clinical picture and with laboratory findings during life. The correlation may be based upon observations on an individual patient, or it may be the result of statistical evaluation of observations on many patients. From several possible answers, the one best suited has to be selected. The conclusion is to be considered tentative, a working hypothesis to be proved by further observations. The pathologist, basing his studies on correlation and integration, thus differs from the basic scientists in his approach to problems and uses the intuition characteristic of any clinical art.

The problem of correlation has become more and more complex as pathology has advanced from the naked-eye observations of the haruspices and the prosectors of the Italian schools to the complex methodology of microscopy, submicroscopy, histochemistry, and histophysics. However, the original challenge confronting Benivieni, Morgagni, Laennec, and Rokitansky has remained the same. It confronts the pathologist in his daily work, in his clinical service, in his teaching, and in his research.

The pathology of the liver offers a timely challenge to correlate functional and structural alterations in view of the coincidence of recent advances in physiology, biochemistry, and anatomy, and the opportunity to supplement autopsy findings with biopsy observations on the liver of living patients. For centuries a big gap existed between the morphologic picture of the organ and the clinical and laboratory manifestations of hepatic insufficiency. This is exemplified by the common experience during autopsy on a patient dead of cardiac failure without any signs of hepatic insufficiency to find an almost complete absence of viable hepatic cells. A correlation between function and structure must exist even if we can not see it. The challenge lies in finding it by proper techniques. One of the earliest leaders attempting to fill this gap was Hans Eppinger in Vienna, who, starting from the morphology of bile canaliculi in jaundice, proceeded to become a leading clinician and who, throughout his entire life, fell back to morphologic study for the evaluation of disturbed function of the liver. Our own attempts to contribute to this problem were stimulated by the inspiration which one of us (H.P.) received in Eppinger's clinic as a young pathologist.

Some of the correlations suggested in this book will possibly be corrected on the basis of further study or improved techniques. Moreover, the coincidence of lesions does not necessarily prove their causal relation. Nevertheless, attempts at correlation, although not removing the necessity for the most thorough morphologic observations, should result not only in better understanding of the basis of morphologic alterations and improved knowledge of hepatic disease, but also in better interpretations of the various hepatic tests.

PART I

Normal Structure and Function

STRUCTURAL PRINCIPLES OF THE LIVER

The liver is composed of small units called "lobules," in the center of which are hepatic vein branches. In circumscribed areas on the periphery of these lobules (portal spaces or tracts), radicles of the portal vein and the hepatic artery are found. These provide blood which flows through the sinusoids toward the central vein. The sinusoids are modified capillaries with specially adapted endothelial cells, the Kupffer cells. Between the sinusoids, the hepatic cells form a continuous framework of plates or sheets. Between the plates and the capillaries are the tissue spaces in which tissue fluid slowly moves. In man, most of this fluid flows toward the periphery of the lobules. The lymphatic vessels start around the blood vessels and nerves in the portal tracts and also in the central spaces. Between adjacent hepatic cells, bile canaliculi are found, through which bile flows toward the portal spaces through collecting ductules. In the portal spaces, these ductules unite and form the smaller bile ducts, which combine to form larger ducts, finally joining at the hilum of the liver in the extrahepatic ducts. Thus, three fluid currents are present in the liver:

1. Blood flowing from the portal space to the center of the lobule
2. Bile flowing from the center toward the periphery
3. Tissue fluids flowing in both directions

The liver contains a number of structural entities, each with specific functions and significantly different types of pathologic alterations, which will be discussed first from the morphologic and then from the functional aspect. These units are (1) the hepatic cell; (2) the Kupffer cell; (3) the blood capillaries; (4) the bile canaliculi; (5) the intrahepatic bile ducts; (6) the extrahepatic bile ducts with the gallbladder; (7) the blood vessels; (8) the lymphatics and connective tissue in the portal spaces continuous with Glisson's capsule; (9) the nerves (Fig. 3).

(8) the lymphatics and connective tissue in the portal spaces continuous with Glisson's capsule; (9) the nerves (Fig. 3).

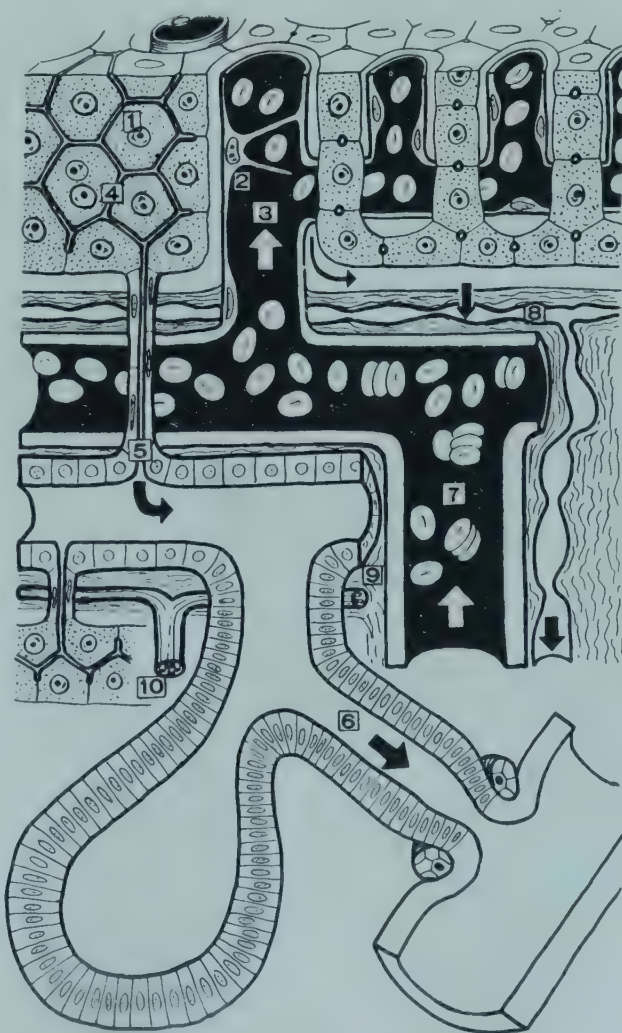


FIG. 3 Basic structures and currents of flow in the liver. (1) hepatic cell; (2) Kupffer cell; (3) sinusoid; (4) bile canaliculus; (5) bile ductule; (6) bile duct; (7) vein; (8) lymphatic vessel; (9) stroma; (10) nerve.

NORMAL STRUCTURE AND FUNCTION

S

The structural units listed are not necessarily functional units. In the kidney, the nephron is a well defined functional unit. In the central nervous system, the functional unit is the neuron. The entire heart acts as a unit. The liver, as the central chemical factory of the body, engages in more diversified activities than any other organ, and

the different functions require different functional units. In a few instances, mitochondria, isolated by ultracentrifugation, or liver homogenates can perform certain chemical tasks. For most metabolic processes the intact hepatic cell is necessary. For some metabolic functions, however, an entire lobule may be needed.

2

METHODS OF STUDY OF STRUCTURE

The structure of the liver can be studied by various methods, which may often give conflicting results. This has created much confusion, and the interpretation of the results and the limitations of the methods require comparison and evaluation. In principle, the methods are (1) gross and microscopic study of form; (2) chemical and physical analysis of (a) microscopic structures (histochemistry and histophysics); (b) isolated cellular elements (cytochemistry); (c) whole organs. The techniques as applied to the liver are better divided into gross inspection, microscopic study, and cytochemical analysis.

GROSS INSPECTION

The conventional gross inspection of the liver has been repeatedly supplemented by injection techniques in which the various vessels of the liver or the bile ducts are injected with india ink or with a 5 per cent solution of gelatin containing such various dyes as prussian blue or carmine [910]. If carmine is used, the vascular tree first should be flushed with dilute acetic acid to prevent diffusion of the dye [2631]. Gross inspection can be supplemented by observation under low magnification in incident light or by inspection of thick frozen sections mounted in glycerin using transmitted light. Preparations in which the vessels are injected with barium can be studied roentgenologically [1535]. In addition, casts of the vessels can be prepared by injecting them with colored Vinylite or neoprene latex and digesting the organ for 4 days in concentrated hydrochloric acid. The resulting specimens can be inspected grossly or microscopically in incident light [2210, 2631]. Vessels smaller than 30 μ usually do not fill. For the study of drug action on vessels, ace-

tone celluloid solutions have been injected when the liver was still functioning and corrosion preparations have subsequently been made [763]. ♦

MICROSCOPIC STUDY

The liver can be studied intravitaly or in tissue preparations by the light microscope, fluorescence microscope, phase microscope, ultraviolet microscope, or electron microscope. From a theoretical standpoint, microscopic examination of the living liver is far superior to the study of tissue sections prepared by conventional methods (paraffin sections). These sections have been subjected to (1) fixation, which denatures the protein and distorts the cellular architecture; (2) embedding, which heats the tissue and removes a large part of its original constituents, leaving a cooked and distorted skeleton of the original. Despite these justified objections to conventional methods of microscopic analysis, neither vital microscopy nor any of the recent improvements in histological technique has produced results comparable to the vast amount of information derived from routine studies on which almost all our information about liver structure is based.

Vital Microscopy

For the study of living tissue in visible light, a fused quartz rod is placed below the area to be studied, to which the rod delivers the light directly [1808, 3463]. Much information on blood flow through the liver has been obtained by this method. To improve visualization of vessels and bile ducts, fluorescent dyes have been injected and their flow has been observed with the fluorescence microscope. Observation of fixed specimens following vital microscopic study has been a valuable

supplement [1242]. The major disadvantage of vital microscopy is the production of artefacts inherent in the observation of living tissue and the necessary temperature control. The studies on cold-blooded animals were initially more successful, but now warm-blooded animals can also be studied.

Examination of Tissue Preparations

During embedding and dehydration the different constituents of the liver sometimes shrink to an unequal degree, and artefacts may develop (Fig. 4, lower part). Artificial slits between hepatic cells and capillaries or between portal connective tissue and lobular parenchyma have been interpreted as preformed tissue spaces. Dry-freezing techniques in which frozen liver tissue is dehydrated in vacuum and then directly embedded in paraffin are, theoretically, the least distorting of all methods [1153]. Various commercial modifications of the original dry-freezing apparatus have simplified the method. However, shrinkage artefacts are readily produced, especially in the initial freezing, and the final slide is not superior to ordinary liquid fixed sections for practical purposes. At present, dry freezing is recommended mainly for histochemical studies in which preservation of all tissue constituents is required. Since removal of the paraffin for staining and the staining itself remove fat- and water-soluble material, embedding media other than paraffin are required to preserve all these constituents in the final section. No such ideal technique exists as yet, reducing the advantage of dry freezing over the conventional techniques. The relative contribution of the different tissue elements can be estimated by histometric techniques [806].

Liquid Fixatives. The choice of a fixative as well as an embedding medium depends upon the specific structures and histochemical constituents to be demonstrated, and quite often compromises have to be made. For routine sections of biopsies as well as autopsy specimens, a buffered or neutralized solution of formaldehyde is the most versatile [1218], because (1) it suffices for almost all diagnostic purposes; (2) the tissue can be kept for an unlimited period of time; (3) least chance of artefacts exists, even if the material is handled by inexperienced technicians; (4) it infiltrates relatively deeply and the specimens can be thicker than with all other fixatives to be listed. The thickness of the specimen should not exceed 3 mm. The cytologic picture and the staining of connec-

tive tissue are not so good as with fixatives containing heavy metals; enzyme stains can not be used and much water-soluble material, such as glycogen, is lost. A better cytologic picture is seen if specimens are fixed for short periods (not over 3 hours for needle biopsy specimen, or not over 16 hours for a larger specimen) in a mixture of nine parts of Zenker solution and one part 40 per cent formaldehyde. After the initial fixation the specimen can be kept in 70 per cent alcohol, but subsequent removal of the mercury precipitate is required. Fixation in Carnoy's solution is recommended for many stains, including those for demonstration of glycogen and pentose nucleic acids and for fluorescence microscopy. Fixation in Carnoy's solution has the disadvantage of hemolyzing erythrocytes. For demonstration of glycogen, absolute alcohol saturated with glucose can also be used. For most enzyme studies and other histochemical analyses, fixation of specimens in chilled acetone is recommended, with initial chilling of the tissue itself before fixation [719, 1218]. Paraffin is best as an embedding medium except where fat-soluble material is to be studied or where special techniques are required. A few investigators prefer celloidin, in which case the sections have to be thicker. For fat stains, frozen sections are used; in the case of small biopsy specimens, previous embedding in Carbowax, 5 per cent gelatin, or a mixture of agar and gelatin is advantageous.

Study under the Light Microscope

For investigation under the conventional light microscope, stained sections are usually employed [2000, 2188, 3392]. Routine hematoxylin (or hemalum) eosin stain is used for most diagnostic purposes. Some laboratories employ phloxine-methylene blue stains.

CONNECTIVE TISSUE STAINS. To demonstrate connective tissue (collagen) fibers and membranes in the liver, Mallory's aniline blue stain is best. The distinct blue color of this stain brings out clearly early distortion of the lobular architecture and increase in connective tissue or membrane. Van Gieson's picrofuchsin stain colors the same elements red but slightly less distinctly. They are also visualized by Gomori's modification of Masson's trichrome stain, which stains the collagen green but less distinctly than the Mallory or Van Gieson techniques [1217]. The trichrome stain aids in the differentiation of granulomas from the epithelial parenchyma. For the demonstration of the

argentaflin reticulum fibers (important in the visualization of collapse and its differentiation from fibroplasia), silver impregnations are used, according to either Wilder or Gomori, in which the black framework, as well as its alterations, stands out distinctly.

FAT STAINS. Fat droplets can be demonstrated in frozen sections of unfixed tissue, or in tissue fixed (preferably for a short period) in formalin, by staining with sudan III or oil red "O" and related dyes. In paraffin sections they are demonstrable after fixation with osmic acid. This causes the fatty acid to become blackened by the reduction of osmium tetroxide to osmium dioxide. In routine paraffin sections the fat appears as vacuoles. Only when these vacuoles are large are they characteristic for fat. However, even in frozen sections the amount of demonstrable fat droplets depends upon the staining techniques, and usually more is found if water-soluble dyes are used.

NUCLEIC ACID STAINS. Unna-Pappenheim's methyl green-pyronin stains pentose nucleic acids (PNA) red, and the nuclear desoxypentose nucleic acids (DNA) green [368, 2059]. This reaction is not specific for PNA, since acidic proteins produce a pyroninophilic reaction [2619], and therefore confirmation is required. One method is the digestion with the enzyme ribonuclease, the crystalline preparations of which are now free of proteolytic activity [1874]. Cold 10 per cent perchloric acid or hot normal hydrochloric acid specifically removes PNA, whereas hot trichloroacetic acid removes all nucleic acids [2474, 2948]. After acid hydrolysis, DNA stains purple with fuchsin-sulfurous acid (Feulgen reaction), supposedly because aldehyde groups are generated.

CARBOHYDRATE STAINS. Rapid hydrolysis of hepatocellular glycogen occurs after death (see Chemical Analysis, later in this chapter). This process comes almost to a standstill within a few hours, probably because of accumulation of organic acids or binding of inorganic phosphates. Liver tissue which has not been fixed shortly after death may therefore reveal a significant reduction or almost complete disappearance of glycogen. Hepatocellular glycogen also disappears in fixatives if they are not strongly alcoholic (absolute alcohol or Carnoy's solution) or if they are not saturated with glucose—in contrast to glycogen in other locations, including the nucleus of the hepatic cell. The frequency with which glycogen can be demonstrated in formalin-fixed material, especially with the periodic acid routine, is surprising. Glycogen can

be stained with iodine, Best's carmine, or Schiff's periodic acid routine (PAS). The PAS stain consists of staining of aldehyde groups with fuchsin-sulfurous acid after oxidation of saccharides by periodic acid. PAS stains replace the older methods for demonstration of glycogen after identification in control sections in which glycogen has been removed by diastase digestion using saliva or commercial enzymes. The PAS also stains red the mucinous secretion of the interlobular bile ducts as a film lining the lumen, wear and tear pigments, fibrin thrombi, amebas and fungi, especially histoplasma, while amyloid shows a poor reaction. The reticulum framework of the liver is less regularly and intensely stained by PAS.

BILE CANALICULUS STAINS. In most species, bile canaliculi can be visualized with Eppinger's hematoxylin stain, Mallory's aniline blue stain, or Gomori's alkaline phosphatase stain.

OTHER STAINS. The attempt to demonstrate bile pigments is still in the experimental stage. Demonstration of elastic fibers assists in visualization of the portal tracts and is helpful in cirrhosis. For cytologic studies of hematopoietic elements, Wolbach's modification of Giemsa stain may be used. Bacteria can be visualized with one of the modifications of the Gram stain, such as Goodpasture's stain. With acid-fast stains, tubercle bacilli can be demonstrated, although only exceptionally, and also wear and tear pigment and ceroid, a pigment occurring in experimental cirrhosis (see Ceroid, under Lipogenic-Lipotrophic Imbalance: Experimental Fatty Liver Cirrhosis Syndrome, Chap. 50). The metachromasia of amyloid can be elicited with either congo red or methyl violet.

HISTOCHEMICAL REACTIONS. Histochemical analysis of various tissue components is a promising field for the future [129, 1218]. Alkaline phosphatase appears black with Gomori's stain, which is the most widely utilized; but, as with all histochemical methods, great care and proper controls must be used to avoid artefacts. Of the minerals, iron, demonstrated with the prussian blue reaction, is the most important. Iron can also be demonstrated by microincineration [2974]. Recently, radioautographs with radioactive substances have been applied [1027].

Supravital Microscopy

Examination of cells from tissue cultures is usually performed after fixation of the specimens [811, 1083]. The distribution of dyes or corpuscular elements is studied after intravenous injection,

primarily to observe the phagocytic activity of the Kupffer cells as part of the reticuloendothelial system. The materials used include india ink preparations [211, 3053], colloidal iron preparations [2445], Thorotrast, Bromsulphalein and other dyes, and lipid emulsions [1621]. Some dyes, such as trypan blue, are stored in both parenchymal and reticuloendothelial cells [695]. Janus green has been injected for visualization of mitochondria. Clearance of injected dyes from the liver tissue has also been considered a test of the functional ability of the cells [3613].

Phase Microscopy

Changes of mitochondria and other cell constituents can be visualized with the phase microscope [3697].

Fluorescence Microscopy

With the simple fluorescence microscope, the distribution of vitamin A, characterized by a green, rapidly fading fluorescence, can be demonstrated in fresh-frozen sections [2625]. This assists in evaluation of the functional study of the hepatic cells and Kupffer cells. Collagen has a bright-blue spontaneous fluorescence, whereas fat can be visualized with the fluorescent stain, phosphine 3R. For the demonstration of porphyrins, which have a red fluorescence, a more elaborate apparatus is required [2932].

Ultraviolet Microscopy

The specific absorption of ultraviolet light by tissue constituents, especially protein and nucleic acid, has been utilized by means of the ultraviolet microscope [512, 2620], with which the distribution of cytoplasmic and nuclear nucleic acids can be studied and quantitatively measured [1898, 1963, 3239].

Electron Microscopy

Only a few electron microscopic studies of the liver have been reported [385], but much progress is to be expected.

CYTOCHEMICAL ANALYSIS

In recent years extensive studies have been performed with ultracentrifugation of the liver. This technique permits the separation of nuclear from protoplasmic elements. The cytoplasm can be subdivided into a large granule layer (0.5 to 2.0 μ in diameter) and a submicroscopic granule layer

the microsomal layer (80 to 150 m μ in diameter). The supernatant fluid above these two layers contains no particles exceeding 20 m μ in size [222, 589]. Liver is the most frequently used tissue for cytochemical ultracentrifugal studies. However, since most of the information has general biologic importance rather than specific bearing upon the problems of the liver function and structure, relatively little reference will be made to this constantly enlarging field.

The supernatant aqueous phase contains the water-soluble albumin, globulins, sugars, pentose nucleic acid, nucleotides and other organic compounds of low molecular weight [589], and some soluble enzymes [16, 368]. It comprises one-third to one-half the total nitrogen or protein present in the hepatic cell [2949]. This layer is rich in potassium and magnesium and poor in sodium and chloride [1917, 2078]. Microsomes contain phospholipids, proteins, and most of the pentose nucleic acid of the cell [2948] and are rich in thromboplastic activity [549]. The microsome fraction has been subdivided into a lipoprotein fraction, in which most of the esterase activity is found, and a nucleoprotein fraction [2487]. The large granule fraction consists of mitochondria and possibly secretory granules [589]. The various large granules can be identified by centrifugation or electron microscopy [589, 590]. The chemical nature of this fraction, which contains many enzymes exclusively, is discussed in the following chapter.

CHEMICAL ANALYSIS

Many substances have been determined in hepatic tissue, usually after homogenization, and small amounts of tissue can now be subjected to analysis. The components most widely studied are (1) water content, determined as the difference in weight of fresh and dehydrated tissues; (2) total lipids, by extraction of liver tissue preferably ground with anhydrous sodium sulfate; (3) glycogen, after dissolving fresh liver tissue in hot 30 per cent potassium hydroxide to avoid postmortal hydrolysis or by using iodine or anthrone reactions on trichloroacetic acid extracts of fresh tissue [3411]; (4) total protein, as the difference between total nitrogen and nonprotein nitrogen in homogenates; (5) various enzymes, the combination of esterase with alkaline phosphatase being advantageous, because esterase levels drop and phosphatase levels rise in liver

damage [2637]; (6) collagen [2351]. Recently, microchemical methods have been applied, especially to biopsy specimens.

The major problem confronting such studies is the question of a reference point, since the water, fat, or glycogen content may vary, and with them the total solid content. Despite extensive discussions, the problem has not been solved

[2625]. In short-term animal experiments, the initial body weight seems to be the most preferable reference point, and for human tissue obtained by biopsies the concentration of desoxypentose nucleic acid (DNA) appears to be best [477, 737]. Even this reference point, however, may be misleading, especially in studies entailing regeneration of liver tissue or necrosis.

3

STRUCTURE OF THE HEPATIC CELL

The smallest unit of the liver is the epithelial hepatic cell, which performs most of the numerous functions of the liver. It has a rather simple structure but is capable of extremely varied functional activities. This cell has morphologic and functional features which are peculiar to the liver, in addition to the characteristics of parenchymal cells generally. The epithelial cell is polygonal in shape, about 30 μ in diameter, and has eight or more surfaces. It does not have a specific cell membrane, but the condensed cytoplasm on its periphery acts as one [2249]. As in any other cell, cytoplasm, nucleus, and nucleoli may be recognized (Fig. 4, top).

ARRANGEMENT OF HEPATIC CELLS

The concept that hepatic cells are arranged in the form of cords was originally presented by Gerlach more than one hundred years ago and was illustrated primarily by Stöhr and Braus. In contrast to the assumption of long cylindrical structures forming the basis of the hepatic parenchyma was the theory of Hering, who, almost one hundred years ago, assumed that the liver of the rabbit represents a continuous mass of cells which is traversed by the sinusoids so that one-cell-thick plates are formed. This concept had been neglected except for brief mention in an occasional textbook. Recently, Elias [907] reexamined the structure of the mammalian liver on the basis of tridimensional reconstructions and found that the hepatic cells are normally arranged in the form of plates, one cell in thickness, which anastomose with each other (Fig. 5). These plates thus form an irregular wallwork which surrounds spaces containing the sinusoids. The shape and size of the hepatic cells conse-

quently vary depending upon the position of the cell within the plates. This description can be better reconciled with the picture actually seen in histologic slides than can the older concept of hepatic cell arrangement.

Where the lobule borders on the portal tract, the hepatic cells are arranged in the form of a one-cell-thick plate, as a sheath enveloping the portal tract. This sheath is called the "limiting plate" [1379]. The cells of the limiting plate are smaller than normal, deeply basophilic, and free of glycogen.

CYTOPLASM

The cytoplasm of the hepatic cell is not homogeneous in histologic sections, and it contains many granules and vacuoles. The appearance is greatly dependent upon the fixative used, and therefore, many of the granules, especially those in routine sections from which fat and glycogen have been removed by fixation and embedding, may be artefacts. Some granules are also found, however, in vital microscopic and dry-frozen preparations. A sharp separation of the particulate and the interparticulate substances is difficult. In histologic sections the interparticulate substance is seen as a fine acidophilic film, apparently of precipitated basic protein [589, 1917], about which little is known. On the basis of fluorescence microscopy, it is thought to contain water-soluble vitamins, such as riboflavin [1503], and water-soluble substances, such as vitamin A alcohol [1192]. In view of the nebulous definition of granules and vacuoles, the cell constituents of known chemical constitution are considered first, followed by a description of various other particles and cell organelles.

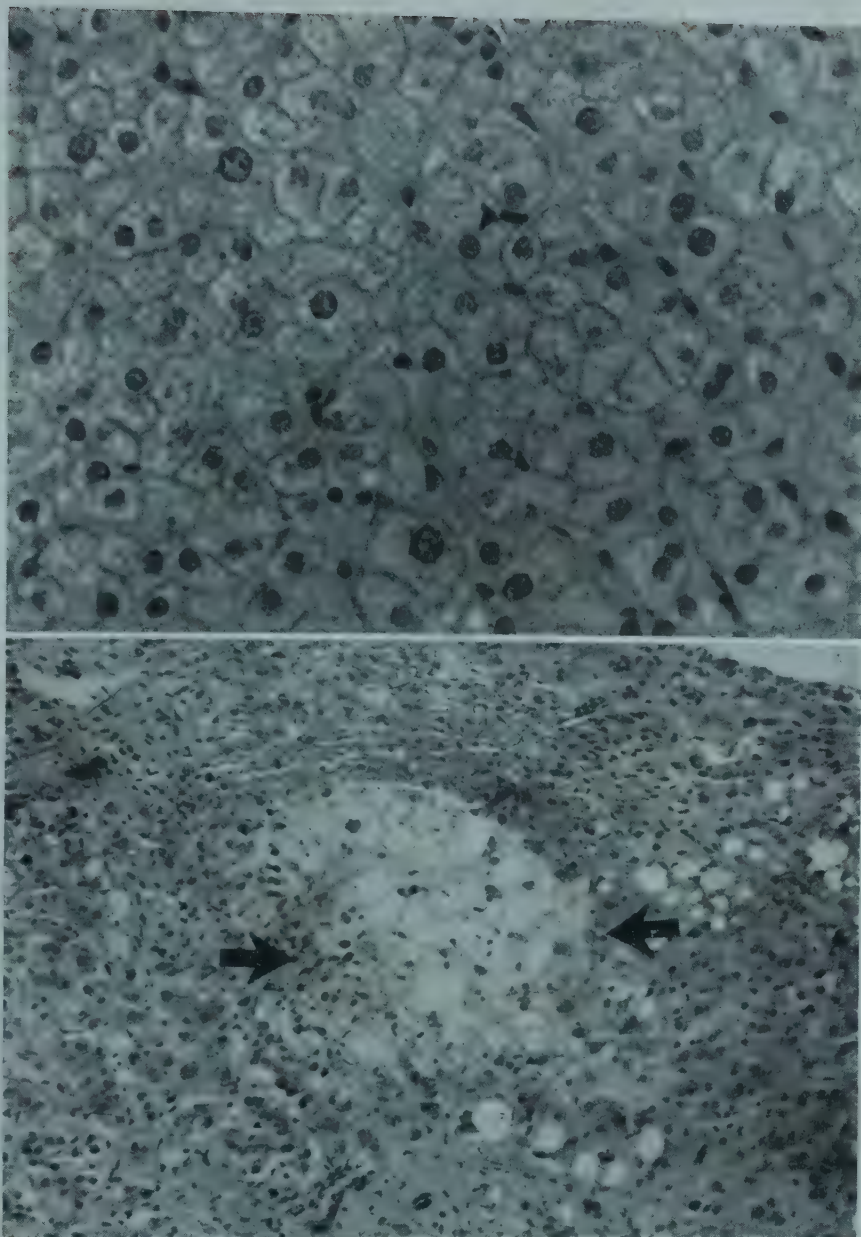


FIG. 4 *Top.* Histologic appearance of normal hepatic cells. H&E ($\times 285$). *Bottom.* Hydropic appearance of hepatic cells in biopsy specimen, due to artefact caused by fixation. H&E ($\times 120$). (Specimen from Dr. E. R. Movitt.)

Proteins of the Cytoplasm. In routine paraffin sections, the cytoplasm of the hepatic cells consists mainly of protein arranged in an irregular network surrounding the other cellular constituents. In most cells, this protein material is acidophilic. In the epithelial hepatic cells some basic portions can be demonstrated with various dyes; for instance, the small basic protein, histone, can be stained in tissue imprints with orange G [3585]. So far, few satisfactory staining methods exist for differential demonstration or for cytochemical analysis of cytoplasmic protein. The spectrophotometric measurement of the Millon reaction for tyrosine is promising [2620].

Some of the proteins in the cytoplasm are assumed to be permanent cell constituents [3571], whereas others are considered to be labile proteins, the amount depending upon the nutritional state [16, 1844, 2959]. The labile fractions disappear upon protein starvation and reappear after replenishment exceeds the minimal requirements [16, 922, 1844]. This concept of labile proteins has been questioned, and the idea has been advanced that protein storage is a function of the entire cytoplasm of the hepatic cell [1844]. The changes found during starvation or protein depletion indicate that all cytoplasmic constituents, including water, fat, protein, enzymes, and

solutes, decrease along with a decrease in cell size [1618]. Only the nuclear proteins are not considered labile [1844].

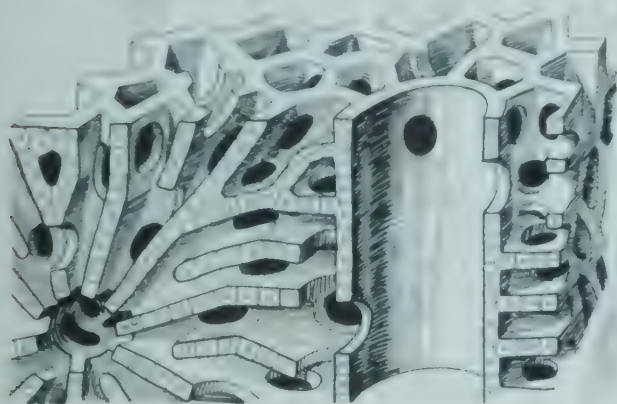


FIG. 5 Stereogram of part of hepatic lobule. The central canal in the lower left corner runs almost perpendicularly to the portal canal. The hepatic plates, extending from the limiting plate around the portal space, end abruptly near the central canal. (From original of Fig. 34, Elias, H.: *Am.J.Anat.* 85:443, 1949.)

During fasting, catalase, cathepsin, succinoxidase, and xanthine oxidase are lost parallel to the loss of liver protein [2294, 1316]. Since the enzyme protein represents a small fraction of the total protein, discrepancies have been repeatedly found. This reduction in enzyme activity is the result of protein loss rather than the result of loss of prosthetic groups. Some enzyme activities have been reported to depend only on the amount of protein [216, 2959].

Pentose Nucleoproteins of the Cytoplasm. Nucleic acids are long chains of nucleotides which consist of a purine or pyrimidine base, phosphoric acid, and a pentose or desoxypentose. They occur in the cytoplasm and the nucleolus, and there are small amounts in the nucleus (heterochromatin) as pentose nucleic acid (PNA) and in the main component of the nuclear chromatin, as desoxypentose nucleic acid (DNA) [737]. PNA has a relatively small molecule and is usually considered less highly polymerized than DNA, although calf and rat liver PNA may have a molecular weight of 300,000, similar to that of DNA [1286]. Both PNA and DNA are bound to protein moieties. Guanine and cytosine are the predominant nitrogen bases, while less adenosine is found than in other tissues [550]. In nongrowing tissue the purine precursors are formed endogenously, particularly from glycine, while in growing tissue exogenous purines are more rapidly incorporated

They are more rapidly incorporated into DNA than into PNA [1109]. The orthophosphoric acid groups of the nucleic acids cause a strong tissue basophilia, which is matched only by the acid mucopolysaccharides. The latter, characterized by strong metachromasia, are not conspicuous in hepatic cells. The basophilia of hepatic cells, as recognized by any of the common basic aniline dyes, therefore results mainly from the PNA compounds. Differentiation from the basophilic DNA of the chromatin is made possible by the red pyronin reaction of the PNA [1887] (Fig. 6, upper left and right), when confirmed by specific digestion with ribonuclease.

Visual observations can be quantitated spectrophotometrically, by measuring either the absorption of ultraviolet rays by the purine and pyrimidine bases of the nucleic acids or the intensity of the pyronin color. Fluorescence microscopy visualizes the loss of PNA; the cytoplasmic protein fluoresces if the quenching effect of the PNA caused by absorption of ultraviolet rays is abolished [3287].

Chemical [1844] and histologic [1898] examinations clearly indicate the dependence of the basophilia on protein nutrition. Choline-deficient diets have no effect on the basophilia [1843], nor does methionine supplementation prevent the loss of PNA during protein depletion [2995]. The ratio of phospholipid phosphorus to nucleoprotein phosphorus is also unchanged by diet or by experimental liver damage [664]. In general, the degree of cytoplasmic basophilia is correlated with nucleolar size, the nucleoli being small in starvation and greatly enlarged in regeneration [1898].

Chemically, the PNA content of the normal hepatic cells somewhat parallels cytoplasmic basophilia. If basophilia is reduced experimentally, a corresponding drop of the chemical PNA content need not occur [976]. This discrepancy indicates that acid-insoluble PNA compounds may still be present even if the basophilia has disappeared, suggesting that for the basophilic reaction the basophilic compound must be in a certain physicochemical state with intact cytoplasmic structure and with proper PNA-protein integration within the molecule.

PNA in the cytoplasm has great biologic significance. It is essential for protein formation, and its presence in the cytoplasm indicates the site of protein formation [369, 512]. The evidence for this is indirect. PNA is found at the sites of greatest protein formation, such as in the pan-

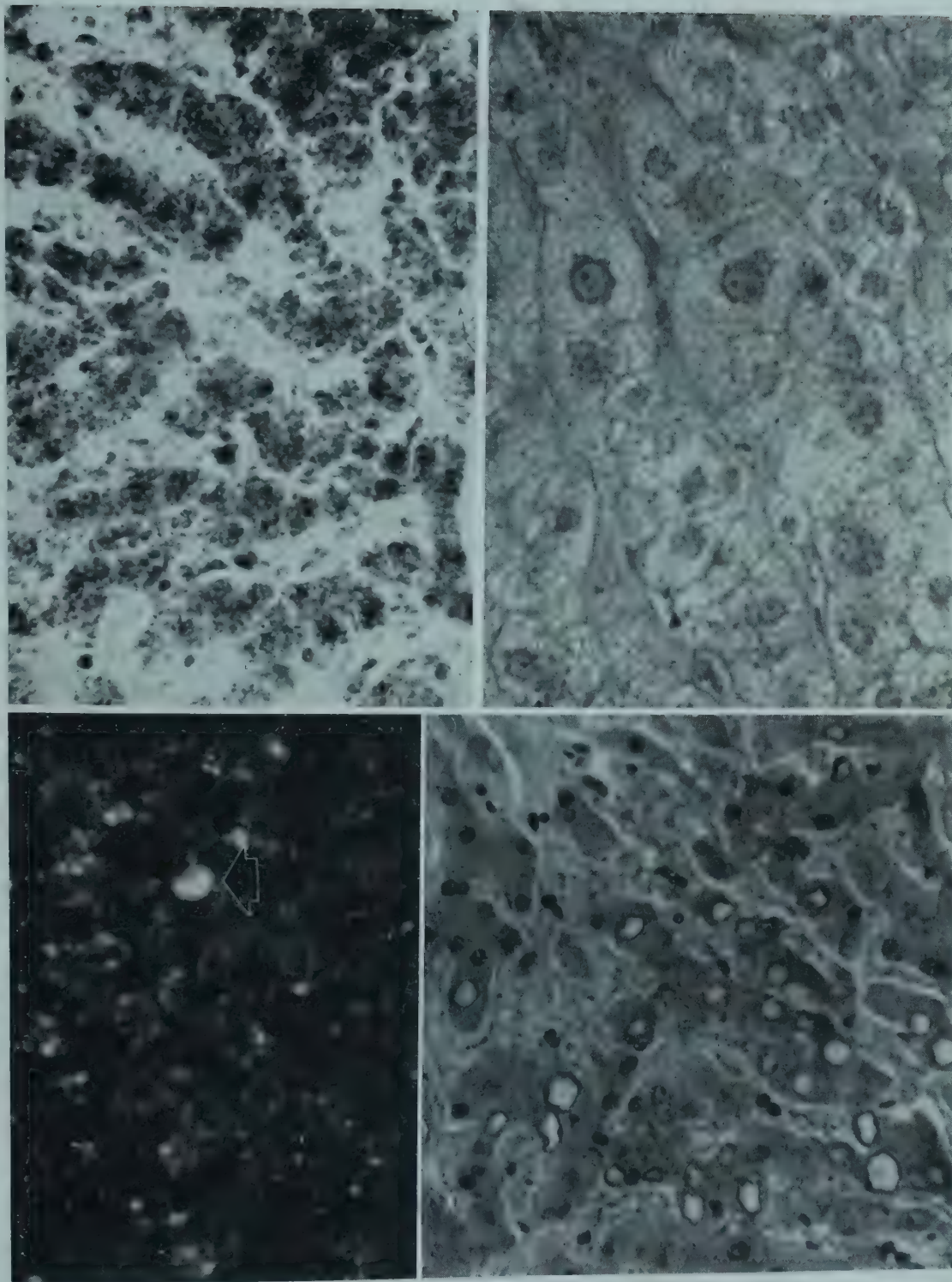


FIG. 6 *Upper left.* Basophilic bodies in normal liver. Methyl green pyronin stain ($\times 350$). *Upper right.* Absence of pyroninophilic bodies in the cytoplasm of a cirrhotic liver. The nuclei are large, and the nucleoli are distinctly pyroninophilic. Methyl green pyronin stain ($\times 450$). *Lower left.* Fluorescence of fat droplets aligned along the edge of the hepatic cell, like beads on a string, and accumulated in the Kupffer cells (arrow). Frozen section stained with phosphine-3R and observed under the fluorescence microscope. *Lower right.* Ballooning of nuclei of hepatic cells due to the presence of glycogen. H&E ($\times 230$).

creas, liver, and Nissl granules of the ganglion cells. The PNA concentration is the highest, furthermore, at the time of greatest protein formation, growth, regeneration, and carcinomatous transformation [1191]. Secretion is also associated with accumulation of PNA in many organs, such as in the pancreatic acinar cells. Various biologic observations have been listed in favor of this causal relation [369], especially the observation that the most significant change during protein synthesis in yeast is the transfer of phosphorus from PNA phosphate, supposedly to provide the energy for protein formation [3155]. Various other findings, however, challenge this intriguing hypothesis of the role of PNA in protein synthesis. X-ray irradiation interferes with the PNA turnover rate without apparent interference with protein synthesis [11]. Furthermore, hepatic cells free of basophilia may be able to form protein [2296]. PNA possibly is present in hepatic cells as a result of protein formation, rather than as its cause, as suggested by the rapid disappearance of PNA simultaneously with labile proteins during starvation or protein deficiency. For the time being, basophilia is considered a histochemical index of the functional capacity of the cells. The protein predominantly formed and released by the liver cells is serum albumin, and a rough correlation between decrease in serum albumin and PNA depletion has been demonstrated [3287].

Glycogen. The liver is one of the main storage sites for glycogen. Under normal circumstances it contains 0.5 to 6.0 gm per 100 gm wet tissue. The amount present depends on the sex of the animal and the composition of the preceding diet [1729]. Almost complete depletion may occur in an otherwise morphologically and functionally normal liver.

In routine sections glycogen always appears in a granular form which has been considered an artefact, owing to precipitation of the diffusely dissolved glycogen by the preceding fixation. Differential centrifugation studies suggest, however, that glycogen may be particulate in the living cell and that its utilization may depend upon the particulate state [1917]. The glycogen distribution varies under normal circumstances in different zones of the lobule [1219]. When the liver is rich in glycogen, it is uniformly distributed. With lesser amounts, it usually predominates in the peripheral zone, since it is apparently first released from the center.

Cyclic diurnal variations have been demon-

strated [1054]. Maximum glycogen deposits are found during the night, when assimilatory processes predominate, and minimum deposits are seen during the day, when secretory processes (bile formation) are at their height. Starvation abolishes the diurnal variation. In the cell, most of the glycogen is located halfway between nucleus and cell membrane. As a result of fixation, all the glycogen may be displaced to one side of the cell postmortally or even extruded from the cell to be seen as an artefact in the tissue spaces.

After fixation in aqueous fixatives, the glycogen-rich hepatic cells, especially in biopsy specimens, exhibit a foamy, finely reticulated appearance in contrast to glycogen-poor cells, which have a denser cytoplasm. This difference is so characteristic that the absence or extreme reduction of glycogen can be recognized in routine histologic sections. Grossly reduced glycogen content is reflected in (1) reduction of the size of the liver; (2) reduced water content, since glycogen is at least partially responsible for binding water; (3) a darker color, since glycogen gives the organ a characteristic pink hue. Functionally, the liver containing insufficient glycogen is less resistant to poisons. The classic experiments of Claude Bernard revealed the increased susceptibility to phosphorus poisoning in the absence of glycogen. Phosphorus poisoning is usually followed by depletion of glycogen, particularly from the periphery of the lobule, which can be prevented by thyroidectomy [2307].

Neutral Fat. The hepatic cell contains neutral fat in two forms: (1) invisible fat, (2) visible droplets, which do not exceed 4 μ in diameter under normal circumstances. Fat may be present even in newborn infants [816].

Fat droplets are normally lined up like beads on a string on the surface of the cell adjacent to the blood capillary (Fig. 6, lower left). They may contain other material, e.g., vitamin A, recognized by its fluorescence, or fat-soluble pigments. The relation of the fat droplets to mitochondria is still not established. The amount and distribution of fat reflect the result of a series of processes involving transport of fat from the depots to the liver, metabolic processes in the liver, and removal of fat from the liver (see Lipid Metabolism, Chap. 5).

Cytoplasmic Granules. Cytoplasmic granules include either chemically defined structures: proteins, nucleic acid compounds, glycogen, and fat;

or morphologically defined structures of unsettled chemical constitution: mitochondria and Golgi apparatus.

PNA GRANULES. In the normal rat liver [752] or human liver [2131, 3287], granules consisting mainly of PNA can be visualized (Fig. 6, upper left and right). They vary in size and shape in different parts of the lobule. In the central zone well-defined elongated rod or comma-shaped structures up to 3 μ in length are seen [2489]. In the intermediate zone these bodies are finer but more numerous. They decrease further in size on the periphery. On the limiting plate, an intense diffuse basophilia is noted.

The basophilic granules are usually arranged around the nuclei [163, 737, 1636, 1898] or around the bile canaliculi, where the granules may show palisading. Infrequently, granules are seen on the periphery of the cell, while the zone between the nucleus and periphery is rather poorly stained because of the presence of glycogen.

The basophilic granules and diffuse cytoplasmic basophilia vary greatly under physiologic circumstances. As early as the beginning of the century, a decrease of pyroninophilic granules in starvation and an increase upon protein feeding were recognized (Fig. 6). The basophilic granules now identified as pentose nucleic acid protein were originally described as storage protein. In human autopsy specimens, PNA granules can clearly be seen in the hepatic cells, but in biopsy specimens they are not well recognized, and conclusions concerning the distribution of PNA from biopsy specimens are equivocal. Cytoplasmic inclusions in hepatic cells of some animals can be produced by injection of certain proteins [3553].

PIGMENT GRANULES. Few, if any, pigments are found in the liver under normal conditions. However, slight alterations related to age, nutrition, changes in hemoglobin metabolism, or administration of various drugs result in deposition of pigmented substances which are histologically apparent.

Endogenous Pigments. Many of these pigments are poorly defined chemically.

LIPOFUSCIN. Lipofuscin (lipochrome, or wear and tear pigment) represents a group of substances which are closely related to one another but differ by their solubility in xylol [2863], which decreases with age. They are lipogenic pigments and partly oxidation and polymerization products of unsaturated fatty acids [1218]. The lipofuscin

pigments give the PAS reaction to a varying degree, stain with aniline dyes such as basic fuchsin, are poorly impregnated by silver solutions, and stain faintly with sudan even after paraffin embedding. Sometimes an easily dissolved lipid shell gives a strong fat reaction. These pigments have a yellowish-brown fluorescence which is not removed by fat solvents [2625]. The abnormal pigment, ceroid (see Ceroid, under Lipogenic-Lipotrophic Imbalance: Experimental Fatty Liver Cirrhosis Syndrome, Chap. 50), is related to lipofuscin. Lipofuscin is increased in experimental vitamin E deficiency [1246] and has been connected with steroid metabolism [2633]. Lipofuscin accumulates when metabolic activities are decreased, as in old age, in chronic malnutrition, or in debilitating diseases. Therefore, lipofuscin has been called "wear and tear pigment" despite its rapid metabolism [129]. The deposition of lipofuscin gives rise to the connotation "brown atrophy," because this usually occurs in shrunken organs. Lipofuscin is mainly a mesenchymal pigment and is especially found in smooth-muscle fibers, as in vessels. In the liver it is also found in Kupffer cells and in the center of the hepatic cells in a ramified form under apparently normal circumstances. It rapidly disappears in hepatic disorders [129].

HEMOFUSCIN. Hemofuscin is not clearly differentiated from lipofuscin and is probably identical with it [847, 1218] and not related to melanin, as has been claimed [3035]. It gives similar reactions to lipofuscin [847], including staining with basic fuchsin [2188]. It occurs in Kupffer cells and also in hepatic cells as light-yellow granules, becoming almost black in some instances. It does not give an iron reaction, although it often occurs in hemosiderin-rich livers [1172] and is considered responsible for the discoloration in hemochromatosis [2187].

A golden-brown granular pigment occurs in the centrilobular zone of almost all normal human livers and disappears in diseases [2657]. Its nature is unknown.

IRON PIGMENTS. Protein-bound iron, in addition to that in hemoglobin or cytochromes, is found in the liver in a soluble, nonparticulate form, ferritin, and in an insoluble, particulate form, hemosiderin or cytosiderin, which may be chemically identical to ferritin. Under abnormal circumstances inorganic iron also appears in particulate form. Ferritin (see Ferritin, under Anabolism, in Chap. 6) contains 23 per cent ferric hydroxide,

and hemosiderin contains 35 per cent. In both instances, the polymerized iron aggregates in the interstices of the same protein moiety, apoferritin. Hemosiderin may represent an excess accumulation of iron, causing ferritin, which is not demonstrable by histologic methods and which does not give the prussian blue reaction, to become the granular brown pigment, hemosiderin. The excess iron in hemosiderin gives the prussian blue reaction [847]. Hemosiderin contains acid mucopolysaccharides, which account for the PAS reaction which mesenchymal hemosiderin gives before iron removal. Parenchymal cells show the PAS reaction only after iron removal [1140]. Hemosiderin granules appear as hard, well-defined, coarse, golden-brown granules in routinely stained sections. They are up to $2\ \mu$ in diameter, usually around the nuclei as well as along the bile canaliculi. They react with acid ferrocyanide, usually at room temperature. Hemosiderin is insoluble in water, alcohol, and alkalis but soluble in acids. It does not give a fat reaction [2188]. The most reliable method for visualization is microincineration and a subsequent prussian blue reaction. In intact sections demonstration of the iron with the Turnbull or prussian blue reaction is not always easy, which explains variations in results obtained by different investigators. Sometimes treatment with hot acids is necessary [2188]. By this method ferritin, which does not appear as particulate material, may cause a diffuse bluish tinge in the entire cytoplasm.

In the normal liver no particulate iron stains with the commonly used iron reactions. After mild autolysis these reactions normally become positive. Without autolysis iron can be seen if its content exceeds 0.1 per cent of the dry weight, which is abnormal.

Cytosiderin is supposedly a mixture of hemosiderin and lipofuscin and is derived from mitochondria [1172]. A black pigment found in frog livers [1865A] gives an iron reaction only after energetic heating or after bleaching with hypochlorite [1172].

Increased iron is demonstrable mainly in the Kupffer cells in viral hepatitis and after blood transfusions and mainly in the hepatic cells in nutritional deficiencies, brown atrophy, fatty metamorphosis, in most forms of cirrhosis, and in pancreatic disease. More iron is found in Kupffer cells than in hepatic cells in various types of anemia and in hemolytic diseases. Excessive iron

is found in the hepatic cells in hemochromatosis. Deposition of large amounts of iron produces a grossly visible brown hue which is termed "hemosiderosis." The morphologic demonstration of increased iron does not mirror the results of chemical analyses (see Iron, under Mineral Metabolism, Chap. 8).

BILE PIGMENT. Bile pigment deposition occurs in all forms of jaundice (see Chap. 21). A precursor of bilirubin, biliverdin, has been demonstrated in phagocytes, in tissue cultures [2804], and in Kupffer cells of geese [2748]. Bile pigment may be present as poorly circumscribed granules, which differentiates them from iron granules. Damaged cells may show a diffusely brown discoloration. Porphyrins are responsible for a reddish-brown, diffuse fluorescence which may be observed in some cases of cirrhosis and which is mainly localized in the periphery of the lobule [1172], usually associated with strong lipofuscin fluorescence [1172]. A similar fluorescence is found in pellagrins.

Exogenous Pigments. Various metallic salts, such as those of silver or bismuth, may be deposited in the liver. Metabolic pigments derived from parasites such as those of malaria or schistosomes should also be listed here. In malaria, some of the pigments may be hematogenous in origin. Another exogenous pigment is carotene, which is dissolved in the lipid portion and contributes to the color of the liver, although it is not a well-defined histologic pigment.

Cell Organelles. MITOCHONDRIA. The nucleic acid-protein compounds combine with phospholipids to form larger aggregates (the large granule fraction) which morphologically correspond to the mitochondria. In the hepatic cytoplasm, the mitochondria are organized structures, microscopically just visible. They are globular in shape in the center of the lobule and rod-shaped in the periphery [752] and vary in length from 0.5 to $2.0\ \mu$. The mitochondria are aligned on the surface of the cell directed toward the blood capillaries. They can be stained with janus green supravitaly, or, after fixation, with phosphotungstic acid, iron hematoxylin, or other hematoxylin. Several biochemically distinct types occur in the same liver [2510]. Hepatic mitochondria usually give a staining reaction for neutral fat and show vitamin A fluorescence. They also contain carotene, which accounts for their yellow color. They can be separated and washed in

saline solution or isolated by homogenizing the liver tissue in 0.88 per cent sucrose [1528]. The mitochondria contain approximately 15 per cent of the pentose nucleic acid [2949] and from 11 to 35 per cent of the total nitrogen [1528, 1952] of the liver. Glycerides, lecithin, cephalin, cholesterol, glutathione, and vitamin A have also been found chemically in the mitochondria [357, 1198, 1528], although vitamin A is possibly only a contaminant [1867]. Mitochondria are thought to be related to the formation of phospholipids and nucleic acids [12, 222, 1726]. Mitochondria contain many enzymes [357], especially those concerned with intracellular respiration [752] such as ATP [2948], cytochrome oxidase and succinoxidase [1527], carbohydrate metabolism [3178], the glucose-glycogen equilibrium, and protein metabolism such as *d*-amino oxidase [589]. The major portions of the ribonuclease and desoxyribonuclease in the liver are found in mitochondria [759, 2949]. The same holds true for the complex enzymatic system of the Krebs cycle [589, 1939], leading to the term "intracellular power plants" [1263].

The hepatic cell contains at least two types of large granules, the mitochondria and the secretory granules, which are either produced by the mitochondria or transformed from them. The many chemically different granules are related to various functions of the hepatic cell [589]. Variations of the state of hepatic function, therefore, alter the fractionation of the hepatic cytoplasm.

Another term for cytoplasmic basophilic granules, cytochondria, has been introduced on a histologic basis, and their morphologic alteration has been connected with hydropic degeneration [2489]. Some cytochondria are mitochondria. They consist of a clear central space and a shell of PNA, possibly as a result of coagulation of nucleoprotein particles on the surface of the mitochondria [392], although this is not supported by studies on isolated, intact mitochondria [1527].

GOLGI APPARATUS. The Golgi apparatus of liver cells is a system of reticular structures which are lipoproteins associated with vitamin C [357]. Its function is apparently concerned with the formation of bile and some of the enzymes in the liver.

It is apparently related to the bile canaliculi [2582] and possibly represents granules which extend as an unbroken chain from cell to cell. The Golgi apparatus has also been related to the

wear and tear pigment found in similar locations [116, 2582].

NUCLEUS

The information about the nucleus of the hepatic cells does not differ much from that about the nuclei of other parenchymal cells [465, 1516].

Chromatin. Chemically, the nucleus contains all the desoxypentose nucleic acid (DNA) of the cell [2949]. All normal cells have a basic mean DNA content of 5.6×10^{-9} mg, although some hepatic cells have exact multiples of this [1963]. DNA is the main constituent of the chromatin, the gene-containing material of the cell. DNA is bound to small proteins, chiefly histones, and it is formed only during cell division. Evidence for this has been derived from studies of the rate of incorporation of radioactive phosphorus into DNA [1474, 3155]. X-ray irradiation depresses hepatic DNA formation [1718]. The relative constancy of DNA, which has resulted in its use as a reference substance, is now challenged [736], although under most circumstances the DNA content per nucleus remains remarkably constant [3332]. The basophilia of the nuclei is not entirely the result of DNA—basophilic proteins are also present. Some PNA exists in the nucleus [822], partly as the "residual chromatin" [2309], insoluble in molar sodium chloride. Its protein component is tryptophane-rich and is not the histone of the chromatin. In the liver this may represent 50 per cent of the chromatin [3174]. The turnover rate of the phosphorus in this PNA is much greater than that associated with other cell fractions [1636].

DNA is a highly polymerized substance consisting of at least 20 nucleotides. Methyl green stains DNA by binding one dye molecule to ten phosphoric acid molecules [1888]. Moderate depolymerization of DNA abolishes the methyl green reaction [1887, 1888] but not the Feulgen reaction (see Nucleic Acid Stains, Chap. 2). Since both lend themselves to spectrophotometric measurements, the ratio of Feulgen positivity to methyl green intensity of the chromatin indicates the degree of depolymerization under pathologic circumstances [1963]. The purine and pyrimidine bases of DNA can be measured by ultraviolet absorption. However, this does not differentiate DNA from PNA. A specific desoxyribonuclease assists in the histochemical differentiation.

Lipids are present in the nuclei [821]. In the dog and rat this amounts to 16 to 18 per cent of the dry weight; one-fourth is neutral fat, while 90 per cent of the remainder is phospholipid, largely lecithin, and some is esterified cholesterol [3603]. Many enzymes are found in isolated nuclei [822], some as a result of contamination with enzyme-rich mitochondria [2949]. High concentrations of specific phosphatases are found in the nuclei [2461, 2948].

Nuclear glycogen is demonstrable by electron microscopy [2346]. Under pathologic circumstances such as congestive heart failure or, more particularly, diabetes mellitus, the nuclear glycogen is increased [1246, 2489] (Fig. 6, lower right). As a result, the nuclei appear enlarged and ballooned, and the chromatin is rarefied. Lipids may be demonstrated in the nuclei with special stains, especially in growing, regenerating, or tumorous hepatic cells [407].

Nucleolus. The nucleus of the hepatic cell contains one or more large nucleoli which stain with pyronin, similar to the cytoplasmic nucleic acids, and which consist mainly of PNA. Ultraviolet spectrophotometric studies have indicated that the nucleolus is optically homogeneous and is exceedingly rich in protein, containing many diamino acids [512]. These studies have also elucidated the function of the nucleolus, which is associated with cytoplasmic protein formation, as is exemplified in growing cells. Chromatin in the center of the nucleus arranged around the nucleolus has been designated "nucleolus-associated chromatin." It produces protein, also rich in diamino acids, which, together with PNA, forms the bulk of the nucleolus. Some of the protein diffuses through the nucleus into the cytoplasm. Outside the nuclear membrane, PNA is formed and combines with the protein from the nucleolus. Simultaneously, the cytoplasmic protein increases (Fig. 7).

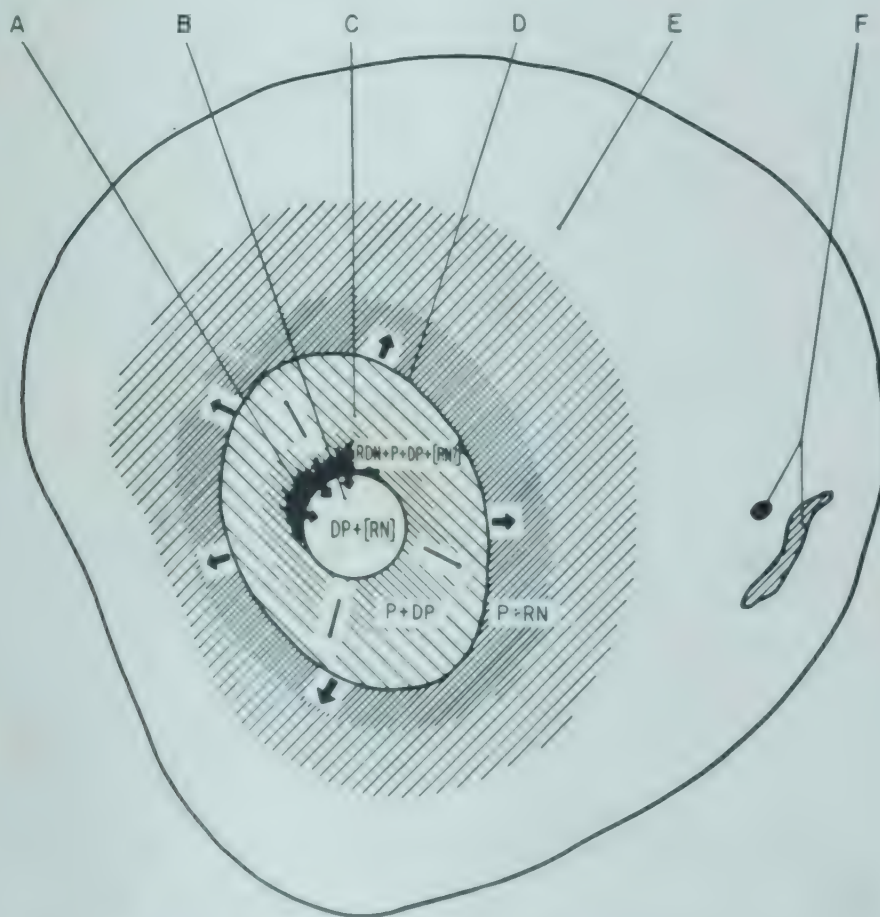


FIG. 7 Diagram of the endocellular system for cytoplasmic protein formation. A, nucleolus-associated chromatin; B, nucleolus; C, nucleus; D, nuclear membrane; E, cytoplasm; F, separate nucleotide-containing systems in cytoplasm; RDN, desoxyribose nucleotides; RN, ribose nucleotides; P, proteins; DP, diamino acid-rich proteins. Brackets mean smaller and varying quantities. (Caspersson, T. O.: *Cell Growth and Cell Functions, a Cytochemical Study*. New York, Norton, 1950.)

Consequently, the increase of the nucleolar mass is a most conspicuous phenomenon during cytoplasmic protein synthesis [512]. Normal hepatic cells have relatively small nucleoli and a lower concentration of PNA in the cytoplasm, while, after partial hepatectomy, the nucleoli enlarge and are rich in PNA which has accumulated in the nucleus around the nucleolus. A similar phenomenon is seen in the livers of rats initially starved or placed on protein-deficient diets and subsequently repleted with proteins [1898] (Fig. 8). During starvation, the nucleolus diminishes rapidly in size and the nucleolus-associated chromatin decreases in amount. The cytoplasmic PNA disappears first from around the nucleus, and then it is lost from the rest of the cytoplasm, while the total nitrogen content of the liver drops [737]. On protein repletion, the nucleolus-associated chromatin reappears, and the nucleolus and then the nucleus enlarge. PNA first appears in the cytoplasm around the nucleus, with an increase of the total nitrogen in the liver, and only later in the remaining cytoplasm. Finally, radioactive phosphorus is incorporated faster into the nuclear PNA fraction than into the cytoplasmic PNA, suggesting that a nuclear component is the source of cytoplasmic PNA [163, 1648]. The size of the nucleolus is an indication of the activity or at least the state of readiness for protein formation. In the human liver, especially in regenerating cirrhosis, large nucleoli and perinuclear cytoplasmic basophilia may be noted in cells otherwise free of basophilia [3287].

This picture suggests protein formation in a depleted liver in which the cells are beginning to regenerate.

Physiologic Nuclear Variations. The nuclei vary in size throughout the lobule, their volume allegedly falling into a geometric series (1:2:4:8). One size usually predominates throughout a lobular zone. In the center and periphery the small forms are found, while the large nuclei are seen mainly in the intermediate zone [153, 1614]. Binucleated cells are frequently seen, especially in younger age groups. They are also very common in regenerating cells (see Regeneration, Chap. 13). The "dark cells" with very granular cytoplasm, smaller than other cells in the vicinity, represent cells disintegrating after normal wear and tear [2582]. Hyperchromatic nuclei are common in senility [73].

Inclusion Bodies. The nuclei of the hepatic cells sometimes contain eosinophilic inclusion bodies, usually sharply demarcated from the surrounding basophilic nuclear rim. They occur in inclusion body disease, in lead poisoning [293], and in viral hepatitis, without any known significance. Glycogen inclusions in the nuclei (see Glycogen, under Cytoplasm, earlier in this chapter) (Fig. 6, lower right) differ from inclusion bodies by the lack of eosinophilia and by a positive reaction for glycogen. If the border of the glycogen vacuole producing ballooning of the nucleus is very sharp, the differentiation from an inclusion body is difficult in a routinely stained hematoxylin-eosin section.

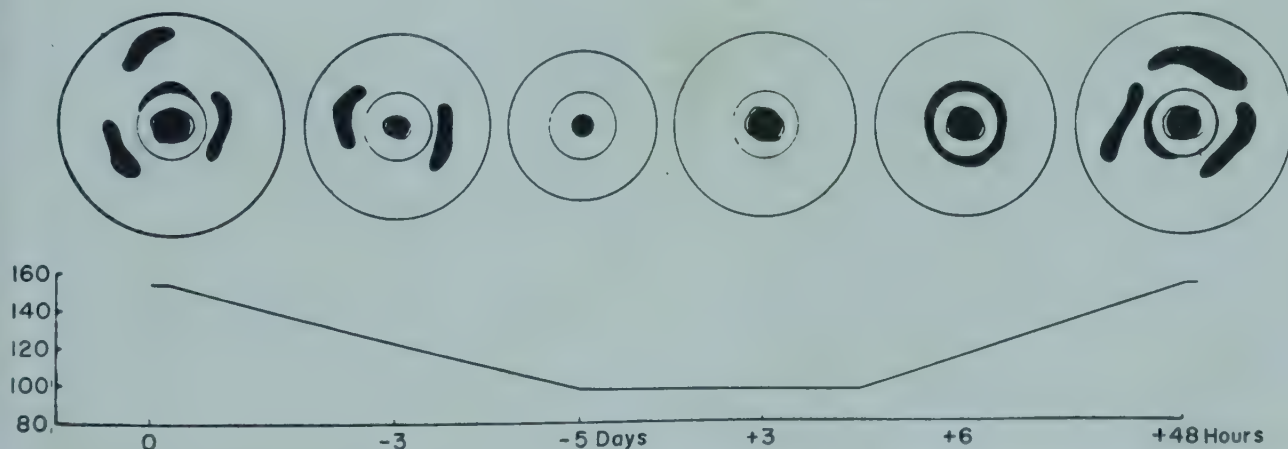


FIG. 8 Graph illustrating the principal changes within the hepatic cells during starvation followed by feeding a high-protein diet. The hepatic cells are represented by the rings. The solid black bodies indicate pentose nucleic acid-containing structures in cytoplasm and nucleoli. The nucleus is represented by a small ring and the nucleolus-associated chromatin by a fine black line around the nucleoli. The diameter of the nucleoli is proportional to the surface of the nucleolar mass measured in the individual experiments. The curve records the changes in the total nitrogen content of the liver. The ordinate scale gives the total nitrogen values in milligrams of nitrogen per 100 gm initial body weight. (Lagerstedt, S.: *Acta Anatomica*, Suppl. 9, 1949.)

METABOLIC FUNCTION OF THE LIVER: THE METABOLIC POOL AND METABOLIC INTERRELATIONSHIPS

The hepatic cell performs storage, anabolic, catabolic, secretory (exocrine and endocrine), and excretory functions. Storage, catabolic, anabolic, and endocrine functions concern individual chemical compounds and are discussed together under metabolic function, divided according to the substances involved. In distinct contrast are those functions of the hepatic cell which result in the formation of bile and which are discussed separately.

In the liver, more than in any other organ, the metabolism of carbohydrates, fats, and proteins is interwoven, in that small metabolites are com-

mon breakdown products as well as common building stones of proteins, carbohydrates, and fats. These small metabolites constitute a metabolic pool. Many varied reactions of degradation and synthesis utilize the same small metabolites, such as acetate, giving rise to the term "common metabolic pathway."

THE METABOLIC POOL

The metabolic pool in the liver serves not only the needs of the liver but those of the entire body as well. Metabolites with one-, two-, or three-

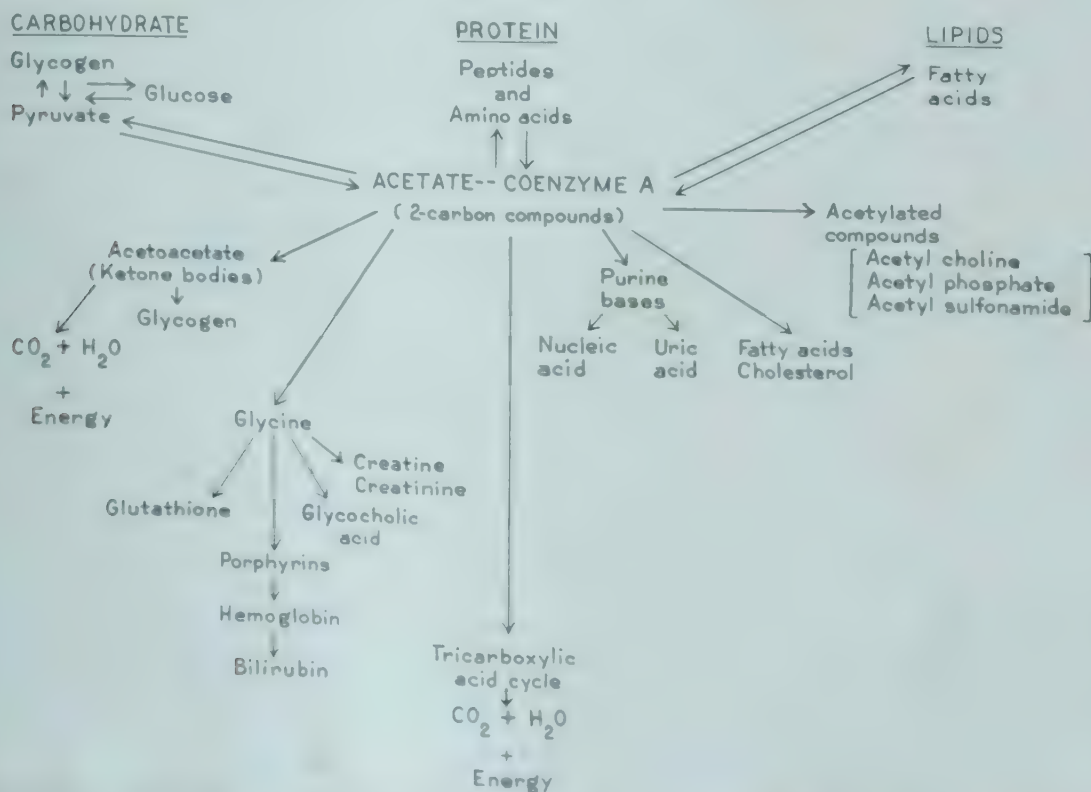


FIG. 9 Metabolic relationships between carbohydrates, fats, and proteins.

carbon atoms, such as formate [2872], acetate [1268, 2595], and pyruvate [2066], serve as a common source for almost all major tissue constituents, including carbohydrates [303], amino acids [1268], fatty acids and glycerol, cholesterol and steroid hormones [2595] (Fig. 9). Acetate, the keystone of these metabolic processes, is never free but in an active form, acetyl coenzyme A, containing pantothenic acid and a high-energy thiol group [2459]. Lipoic, or thioctic, acid is necessary for the formation of acetyl coenzyme A by oxidation of pyruvate or alpha-ketoglutarate, and it also contains a high-energy thiol bond [3424]. In its active form it is combined with thiamine pyrophosphate.

Glycine, also formed from acetate, plays a key role in the formation of nitrogenous substances such as nonessential amino acids, purine bases for nucleic acid, and pyrroles for hemoglobin formation. Amino acids other than glycine may directly enter the pool without passing through an acetate form. Some of these processes are restricted to the liver.

ENERGY PROVISION

Large amounts of energy are required for the metabolic transformations in, to, and from the metabolic pool. This energy comes from two principal sources. One is the nucleotide, adenosine triphosphate, or ATP, which rapidly releases energy when it becomes adenosine diphosphate, or ADP, by losing a high-energy phosphate bond. The second source is the aerobic oxidation of substances in the metabolic pool to carbon dioxide and water via the tricarboxylic acid cycle of Krebs. The main purpose of the second source is to restore the high-energy phosphate bonds to re-form ATP by a series of metabolic transformations. Active acetate enters the Krebs cycle by combining with oxalacetate to form citrate, which in turn is converted to alpha-ketoglutarate, succinate, fumarate, malate, and back to oxalacetate. In this process the two-carbon atoms of the acetate are oxidized to carbon dioxide and water. The cytochrome enzymes, ATP, magnesium ions, and pyridine nucleotides are important cofactors in the Krebs cycle. A small amount of any one of the intermediates is needed to act as a "spark" for the reaction [1263, 1939]. This so-called "cyclophorase system" [1263] is located chiefly in the mitochondria of the hepatic cell in the liver [1939].

Relation of Carbohydrates to the Metabolic Pool. The breakdown of carbohydrates, especially glycogen, to the metabolic pool in the liver varies from that in other organs. The liver utilizes little hexose via classic glycolysis to pyruvate, which occurs in other tissues, because the necessary phosphohexokinase enzyme system is relatively inactive in the liver [2482]. An alternative pathway, a hexose monophosphate shunt, accounts for 75 per cent of the glucose utilized by the liver [316]. The terminal carbon atom of glucose phosphate is oxidized, with the formation of phosphogluconic acid and subsequently of the pentose, ribose, which the liver uses in nucleic acid synthesis, or which can be broken down to pyruvate and then to the metabolic pool [789]. The liver forms glycogen not only from its own metabolites but also from breakdown products in the blood, such as lactic acid in the Cori cycle [661] (Fig. 10).

Relation of Lipids to the Metabolic Pool. Most of the natural fatty acids contain long carbon chains with an even number of carbon atoms, usually 16 or more. Fatty acids are derived mainly from neutral fat, phospholipids, and cholesterol esters, with the help of esterases. The glycerol portion of the lipids is either converted to hexose or broken down to smaller products which enter the metabolic pool. Choline and inositol from phospholipids and cholesterol are also metabolized, with formation of acetate.

Fatty acids are oxidized on the second carbon atom following the carboxyl group and the terminal two-carbon atoms disposed of as acetate. The fatty acid molecule is completely broken down by beta oxidation into many two-carbon chains, which may combine with each other or with other two-carbon atom metabolites at random [535] to form acetoacetic acid with the help of coenzyme A [3424]. The acetoacetic acid may form beta-hydroxybutyric acid and acetone; the former reaction is reversible, but acetone formation is not. These ketone bodies are formed only in the liver, but the liver has little or no ability to oxidize them (see Ketogenesis, near the end of this chapter). However, the breakdown of fatty acid has been demonstrated in various tissues such as kidney, muscle, and testis.

Long-chain fatty acids are formed from acetate, acetoacetate, and acetaldehyde chiefly in the liver [376, 3215]; small amounts are formed rather slowly in the fat depots [3371]. Saturated fatty acids are formed twice as rapidly as unsaturated ones [2595], and both are formed more rapidly

than phospholipids and cholesterol. Fatty acid synthesis, as well as oxidation, requires coenzyme A, which also serves in the formation of acetoacetate and cholesterol [1308] via acetoacetate, acetone, isoprene, and squalene [3424]. Fatty acid synthesis is stimulated by cystine [3370] and by some members of the vitamin B complex, especially thiamine [2125]. The ability of the liver to form fatty acids from acetate is directly proportional to its glycogen content [1416].

Relation of Proteins to the Metabolic Pool.

Proteins are condensation products of a large number of amino acids, which vary in arrangement and amount in different proteins. The proteins are broken down in all tissues by intracellular and extracellular proteolytic enzymes to polypeptides and amino acids. The metabolic fates of the amino acids vary. Some of them enter the metabolic pool through a variety of processes. The formation of amino acids requires an amino group, the source of which may be ammonia or other nonamino nitrogenous compounds (amination), or it may be another amino acid (transamination) with the amino donor returning to the metabolic pool (deamination). Some amino acids are not formed in sufficient quantities and are

essential dietary constituents. In man these are valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine, and tryptophane.

AMINATION. Amino acids formed by combination of ammonia with products of the metabolic pool include glutamic acid formed from alpha-ketoglutaric acid [1862]. Experiments with isotopic nitrogen show that the ammonia nitrogen may be incorporated to some extent in all nitrogenous compounds of the body, including most of the amino acids, except lysine.

TRANSAMINATION. Glutamic acid reacts with pyruvic acid in muscle, heart, brain, liver, and kidney to form alpha-ketoglutaric acid and alanine after exchange of the amino group between the two molecules. Transaminase enzymes containing pyridoxine have been isolated. Originally only aspartic acid, alanine, and glutamic acid were thought to be formed by transamination with the help of dicarboxylic and tricarboxylic acids of the Krebs cycle [608], but now many other amino acids are thought to be formed by the same route [470, 1895].

DEAMINATION. Amino acids lose the alpha amino group by oxidation owing to the action of amino acid oxidases, most of which contain riboflavin

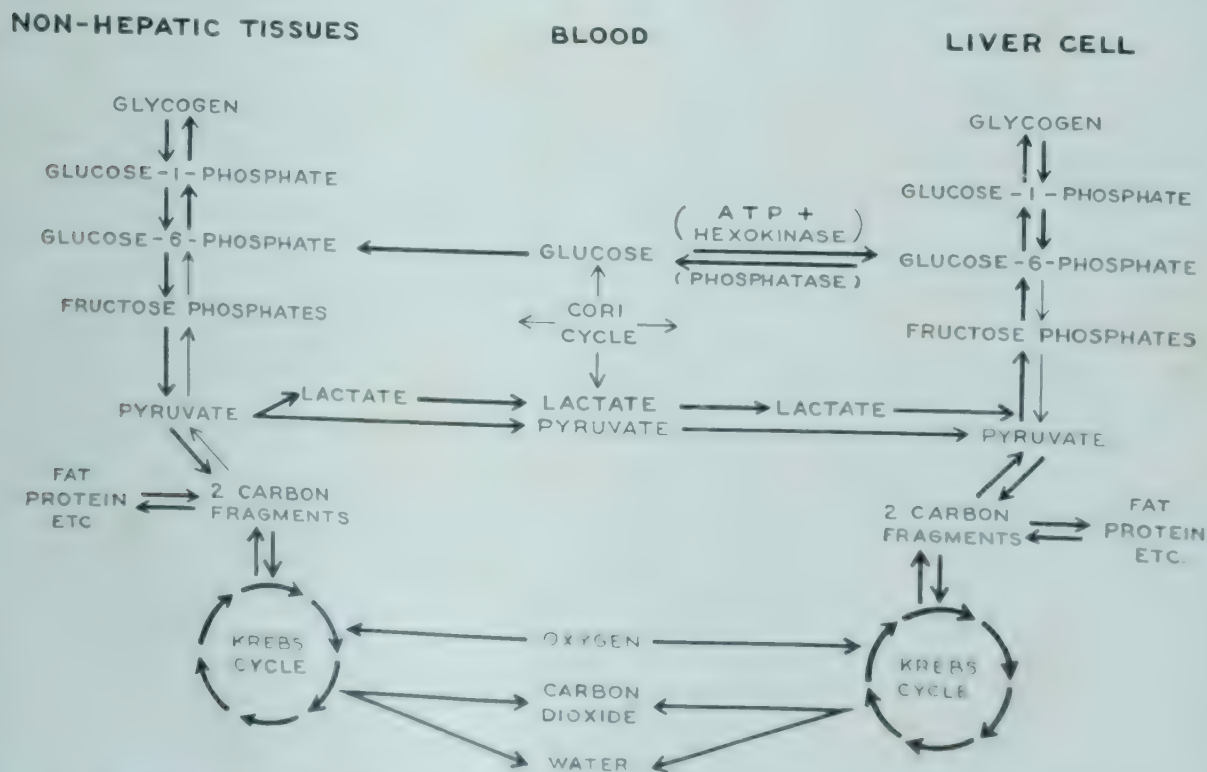


FIG. 10 Metabolism of carbohydrates in hepatic cells and nonhepatic tissue, and the relation to the metabolic pool. The Cori cycle is the chiefly unidirectional movement of glucose to the peripheral tissue, with its return to the liver as lactate or pyruvate. The heavier arrows indicate preferential routes. The hexose monophosphate shunt is omitted.

and are present in liver and kidney [1263]. The resulting keto acids enter the metabolic pool.

GLUTAMINE BREAKDOWN. Amino groups formed in the peripheral tissues combine with glutamic acid to form glutamine, which is removed from the blood stream by the liver. The second amino group is converted to ammonia in the liver and transformed to urea, which is excreted by the kidney. Therefore, in hepatectomized animals the blood-urea level is low and the glutamine level is high. The blood-ammonia level appears high [339], but this results mainly from *in vitro* hydrolysis of glutamine after the blood has been collected.

KREBS-HENSELEIT CYCLE. The metabolism of ammonia and urea has been clarified by the description of the Krebs-Henseleit cycle, which occurs only in the liver in mammals [1863]. The specific enzyme involved, arginase, is found only in hepatic cell mitochondria. This enzyme splits arginine into urea and ornithine. The ammonia liberated by the deamination process of other amino acids combines with ornithine and carbon dioxide to form citrulline. The citrulline unites with another molecule of ammonia derived from aspartic acid to form arginine.

FATE OF GLYCINE. Glycine (aminoacetic acid) undergoes more metabolic transformations than any other amino acid. Without deamination it serves in the formation of porphyrins, hemoglobin, bilirubin [2690], glutathione [1648], creatinine and creatine, and other amino acids such as serine [2871] and aspartic acid [3161]. It may be transformed to purine bodies, which form uric acid, during which process the C—C—N linkage may be preserved. Glycine also combines with bile acids to form glycocholic acid. It serves in detoxification processes; for instance, in the formation of hippuric acid and other substances (see *Conjugation of Bile Acids*, Chap. 9, and *Conjugation*, Chap. 10). It also enters the metabolic pool as acetate [3161].

PROTEIN SYNTHESIS. Protein is synthesized through the polypeptide binding of amino acids. Amino acids are made available by intestinal absorption, by breakdown of tissue protein, and by synthesis from the metabolic pool. The energy required for this synthesis is derived from ATP and the Krebs tricarboxylic acid cycle. This has been demonstrated for the synthesis of glutathione, a tripeptide formed *in vitro* from glycine, cystine, and glutamic acid by liver homogenates [1648]. The common metabolic pathways thus

make possible the formation of protein from fat and carbohydrate.

TRANSMETHYLATION. In recent years the great importance of the methyl radical as a dietary and metabolic factor has been emphasized [868]. Originally, it appeared that methyl groups can not be formed and were transferred from one compound to another. Choline and methionine appeared to be the most important methyl donors to be provided by food intake or tissue breakdown [868]. The direct transfer of a methyl group from choline to homocysteine to form methionine (transmethylation) occurs when these substances are incubated with liver homogenates [850]. Some methyl groups can also be formed in the body from small carbon compounds of the metabolic pool such as formate [869, 2872], under the influence of vitamin B₁₂ [869]. This process probably occurs only in the liver.

METABOLIC INTERRELATIONS OF FATTY ACIDS, CARBOHYDRATES, AND PROTEINS

From the interrelations presented, proteins, fats, and carbohydrates appear to be potential precursors of each other through the common metabolic pathways of the metabolic pool in the liver. The ultimate energy requirements of the liver are met by glycolysis, mainly via the hexose monophosphate shunt, and by the oxidation of two- and three-carbon atom metabolites in the metabolic pool.

Gluconeogenesis. The transformation of fatty acids and protein to carbohydrates occurs predominantly in the liver and is called "gluconeogenesis." First, glycogen is formed, from which glucose is ultimately obtained. This transformation provides an important source of blood sugar beyond that directly available from glycogen stores and from hexoses absorbed from the intestine. The significance of gluconeogenesis has been primarily tested in diabetes.

In starvation, after disappearance of the glycogen depots, usually after 12 hours, gluconeogenesis begins.

Although gluconeogenesis from fat was questioned, tracer experiments proved that fatty acids, including palmitic acid [681, 2066, 3252], can provide the building stones of glycogen in the liver, although the extent of this process may be limited.

Gluconeogenesis from protein has been demonstrated on the basis of (1) quantitative increase

of glycogen in starved animals; (2) the excretion of glucose in the phlorhizin-intoxicated animal in which gluconeogenesis is stimulated by glucose loss in urine; (3) experiments with tagged amino acids. The following amino acids appear to form glycogen: glycine [2481], alanine [2133], serine, valine [552, 2809], the dicarboxylic amino acids, proline and hydroxyproline, cysteine and cystine [1472], and histidine [987].

Formation of Fatty Acids from Carbohydrates and Proteins (Lipogenesis). The transformation of glucose to fatty acids takes place in the liver at a far higher rate than in other organs examined [561], including fat depots [790]. It requires considerable energy, because fatty acids have a higher caloric value than glucose. The chemical processes probably take place via acetate and acetoacetate with the aid of coenzyme A. Acetate is used for fatty acid formation by cell-free homogenates of liver or by a water-soluble enzyme system derived from that organ [376]. Storage in the form of fat exceeds glycogen storage when excessive amounts of glucose are administered [3214] and may result in the development of fatty metamorphosis of the liver [2238].

Since deamination of amino acids to short-chain fatty acids occurs mainly in the liver, this organ

is the main site of fatty acid formation from protein. Ketone bodies are formed from leucine [607], phenylalanine, and tyrosine [3544] and can then be used as building blocks of fatty acids. In this respect, proteins are ketogenic [453, 607, 3544]. Serine is used in the formation of the phospholipid, cephalin [3213].

Formation of Protein from Carbohydrates and Fatty Acids. This occurs predominantly in the liver, although many tissues, at least in newborn mice, can form several amino acids from glucose [3632]. Individual amino acids, such as alanine, can be formed through transamination (see Transamination, above).

The small metabolites of the fatty acids may be aminated to form amino acids used in protein formation.

Ketogenesis. The relation between fatty acids, proteins, and carbohydrates is reflected in ketogenesis. The ketone bodies acetoacetic acid, beta-hydroxybutyric acid, and acetone, which arise in the liver during the metabolic breakdown of fatty acids, are further oxidized to carbon dioxide and water by almost all extrahepatic tissues, although the liver itself cannot oxidize them [2255]. Exogenous protein, as well as carbohydrate, is anti-ketogenic, despite the fact that ketone bodies can

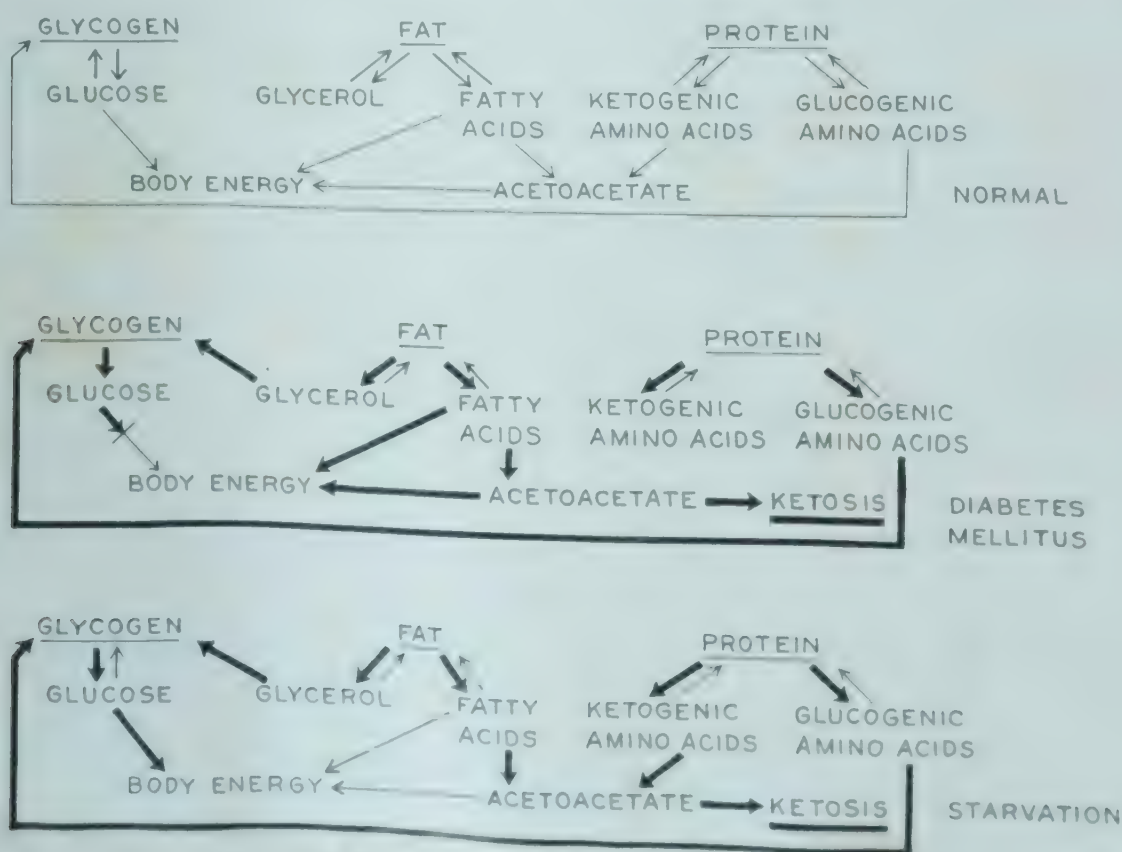


FIG. 11 Pathways of ketone body formation and ketosis

be formed from some amino acids while others, such as methionine [1472] and lysine, are neither ketogenic nor antiketogenic.

KETOSIS. Ketosis, or the presence of excessive amounts of ketone bodies in the blood with associated ketonuria, is the result of an imbalance between formation of ketone bodies in the liver and their oxidation in the peripheral tissues, increased formation in the liver being the more important factor (Fig. 11). In the normal liver, at least in the rat, ketogenesis is minimal and becomes quantitatively important only in the presence of disease or abnormal dietary conditions [3544]. Absence of available carbohydrates or im-

pairment of carbohydrate utilization, as in diabetes, shifts the supply of peripheral energy donors from carbohydrates to the ketone bodies. Notwithstanding the excessive catabolism of ketone bodies in diabetes, the formation far exceeds the utilization, which explains the ketosis. Similarly, in starvation, the utilization of fat from the periphery and the increased breakdown of endogenous protein to replace carbohydrates as energy donors result in ketosis.

In dogs with severe liver damage, the formation of ketone bodies is inhibited even in the presence of starvation or diabetes [3139]. In man, ketosis may be found in liver disease.

5

METABOLIC FUNCTION OF THE LIVER:
CARBOHYDRATE AND LIPID METABOLISM

CARBOHYDRATE METABOLISM

Glucose is the main supply of energy for the body and is transported to the cells as blood sugar. Since all the glucose of the blood originates from the liver in the fasting state, the liver plays a predominant role in carbohydrate metabolism of the body. The importance of this is reflected in the sacrifice of almost all other functions of the liver for the production of blood sugar in liver damage. The liver is the first organ to receive the monosaccharides absorbed from the intestine. The liver transforms, via phosphorylation and with the help of branching enzymes, all hexoses to glycogen, a polysaccharide. The liver also transforms some of the metabolites of protein and fat to glycogen. Other tissues, chiefly muscle, supply glycogen-breakdown products, such as lactic acid, to the blood. The liver ultimately reconverts these products to glycogen and then to glucose in the Cori cycle. Consequently, the role of the liver in carbohydrate metabolism consists of an anabolic formation of glycogen from monosaccharides and other metabolites, a catabolic glycogen breakdown to glucose for release to the blood, and a metabolic conversion of sugar to protein and fat, and vice versa. The liver also metabolizes other carbohydrates, such as pentoses.

Glycogenesis. The sugars reach the liver chiefly through the portal vein, the blood-sugar concentration of which rises to much higher levels than the systemic venous blood-sugar levels in the post-absorptive phase. The ability of the liver to take up glucose from the blood greatly exceeds its ability to release it. For the synthesis of glycogen, glucose is combined with phosphate derived from ATP with the help of hexokinase to form glucose-6-phosphate. The glucose-6-phosphate is trans-

formed to glycogen with the help of a branching enzyme after passing through glucose-1-phosphate with subsequent loss of inorganic phosphorus [2020]. The energy required for the condensation is derived from the high-energy phosphate bonds of ATP.

The anabolic phase is under the influence of hormones. Insulin is necessary for the transfer of glucose across the cell membrane into the hepatic cell (see Effect of Insulin on the Liver, under Relation between the Pancreatic Islands and the Liver, Chap. 62). The hexokinase system is inhibited by anterior pituitary extracts, and this inhibition may in turn be inhibited by insulin.

The liver forms glycogen more readily from fructose than from glucose (see Fructose and Galactose Metabolism, later in this chapter) [661, 2266, 3546]. Galactose is less readily transformed than the other two [661]. D-Lactate is converted to glycogen to a greater extent than the hexoses [661]. Administration of potassium ions decreases glycogen stores without increasing the blood glucose [2990].

Glycogenolysis. The reversible process of glycogen-glucose formation represents part of a cycle which makes glucose available primarily for maintenance of the blood-sugar level. Glycogen is transformed to the Cori ester, glucose-1-phosphate, with the help of debranching enzymes, phosphorylases, and inorganic phosphates [661] (Fig. 10). The Cori ester is changed to glucose-6-phosphate by phosphoglucomutase. The large amount of specific phosphatase present in the liver splits this ester to phosphates and glucose, which is released to the blood stream [629]. Some of the glucose phosphate undergoes glycolysis in the liver to pyruvate, which contributes to the energy

supply of the organ. The hexose monophosphate shunt is the pathway for 75 per cent of hepatic glycolysis, while the classic pathway utilized by all other tissues accounts for the remainder.

Glycogenolysis is regulated by the level of the blood sugar and by hormones. Adrenal cortical extract [2982] and insulin [661, 3139] have an inhibitory effect. Stimulation of the thyroid or adrenal medulla and splanchnic nerves, as well as anemia and acidosis, promotes glycogenolysis [2053, 2572]. Glucagon, the pancreatic hyperglycemic factor, increases hepatic glycogenolysis by activating the phosphorylating enzymes [1740] (see Glucagon, under Relation between the Pancreatic Islands and the Liver, Chap. 62).

Homeostatic Regulation. Hepatic glycogen and blood sugar are in equilibrium (homeostasis) [3139]. When the blood-glucose level rises above a threshold of approximately 120 mg per 100 ml, glucose release from the liver diminishes and glycogen synthesis increases. On the other hand, when the blood-glucose level falls, glycogen synthesis is reduced and more glucose is released to the blood. By comparison of the glucose concentration in the portal and hepatic veins and hepatic artery, and by measurement of the blood flow through these vessels, the liver can be shown to release glucose to the blood in fasting dogs; whereas the liver takes up glucose after intravenous administration until the blood sugar falls below its original level. Thus, the blood-sugar level appears to be the stimulus for the direction of the catabolic-anabolic glucose-glycogen cycle. In this way, the nutritional intake of sugars, in addition to the energy requirements of the body (which influence the extrahepatic glucose utilization), determines the gradient of glycogen formation and the size of the glycogen depot. Of the chemical factors influencing the cycle, the most important is the availability of inorganic phosphate. Salicylates retard the deposition of liver glycogen and lower the blood-sugar level, apparently by interfering with oxidative phosphorylation [3100].

The regulatory mechanisms of the body influence the direction of the cycle. Autonomic nervous stimuli are of importance. Injury to the floor of the fourth ventricle produces glycosuria in well-fed animals, probably by release of glucose to the blood, an effect which is prevented by section of the splanchnic nerves. Hormonal influences upon the catabolic and anabolic phases of the cycle have been mentioned. Some hormonal effects are com-

plex, especially those from the anterior pituitary (see Pituitary Gland, Chap. 62) and the pancreatic islands (see Relation between the Pancreatic Islands and the Liver, Chap. 62).

Blood Sugar. Since the blood sugar is almost entirely formed by the liver, one of the earliest effects of hepatectomy is hypoglycemia [2202]. The rate of blood-sugar formation by the liver in dogs [2981] and in man is about 2 gm per hour, and this formation ceases promptly when glucose is given intravenously. The hepatic glycogen stores are not the only source of the blood sugar, which otherwise would rapidly disappear in starvation when these depots are exhausted. The amount of glycogen that can be formed from lactic acid coming from the peripheral tissues is also insufficient. Gluconeogenesis from pentoses, amino acids, and fatty acids via glycogen occurs in the liver to supply blood sugar when other available sources are depleted. In human liver disease this auxiliary mechanism, even in severe hepatic damage, prevents hypoglycemia in the absence of hepatic glycogen stores. The disturbed release of blood sugar in liver disease is recognized by (1) a diabetic type of glucose-tolerance curve; (2) glycosuria after intravenous glucose administration; (3) a decreased response of the blood sugar to the administration of epinephrine; (4) a tendency to fasting hypoglycemia, which is rarely seen clinically.

Fructose and Galactose Metabolism. **FRUCTOSE.** A large portion of injected fructose is taken up by the splanchnic bed, mainly in the liver, and is converted to glycogen or to lactate or pyruvate [2266]. Fructose is phosphorylated in the liver by a specific fructokinase with the help of ATP to fructose-1-phosphate. This is either broken down to pyruvate and lactate, which enter the metabolic pool, or transformed by an isomerase to glucose phosphate and then eventually to glycogen. Although these reactions require more enzymatic steps and more ATP than the formation of glycogen from glucose, glycogen formation from fructose is more rapid than from glucose [663, 1469, 2266].

GALACTOSE. Galactose split from the lactose in ingested milk in the intestine is phosphorylated to galactose-1-phosphate by galactokinase with the help of ATP. The phosphorylated compound is transformed mainly in the liver into glucose-1-phosphate by phosphogalactoisomerase, a Walden inversion enzyme, in the presence of a coenzyme, uridine diphosphate glucose. The glucose phos-

phate subsequently participates in glycogenesis and glycogenolysis [2777, 3351].

LIPID METABOLISM

The liver plays a central role in the metabolism of neutral fats, phospholipids, and cholesterol. It controls intestinal absorption of fats by its secretion of bile acids into the bile. It synthesizes and stores various lipids (Table 1), forms serum lipids,

Table 1 Distribution of Lipids in Beef Liver

	<i>Per Cent of Dry Weight</i>
Total lipid.....	20-25
Neutral fat.....	5.5-6.0
Free cholesterol.....	0.4-0.5
Cholesterol ester.....	0.4-0.6
Phospholipid.....	14-19
Cephalin.....	5.0-8.5
Lecithin.....	8.0-10.0
Sphingomyelin.....	0.7-0.8

Source: Data from M. Kaucher, H. Gallbraith, V. Button, and H. H. Williams: Arch.Biochem. 3: 203, 1943.

aids in the distribution of lipids throughout the body, and is the sole source of the ketone bodies formed from fatty acids which may serve as a fuel for the other body tissues.

Fatty Acids

Fatty acids are constituents of neutral fats, phospholipids, and coupled alcohol esters, such as cholesterol and vitamin A. The liver synthesizes fatty acids, breaks them down, esterifies, stores, saturates, and desaturates them, and removes them from and releases them to the blood. The removal of fatty acids from the liver has been broadly termed "lipotropism." Since it concerns neutral fats, phospholipids, and cholesterol, it is discussed separately.

The liver synthesizes both saturated and unsaturated fatty acids from carbohydrates, amino acids, and other metabolites via the metabolic pool (see The Metabolic Pool in Chap. 4). Long-chain fatty acids can be transformed into one another without breakdown or resynthesis.

SATURATION AND DESATURATION. The liver has the highest concentration of biologically important unsaturated fatty acids, which it absorbs selectively from the blood [3082]. The liver is able to desaturate saturated fatty acids by means of the

enzyme, fatty acid dehydrogenase [3577]. In man, only one double bond can be formed, and therefore highly unsaturated fatty acids, such as linoleic, linolenic, and arachidonic acid, can not be synthesized and are considered essential. The liver also saturates unsaturated fatty acids to a limited degree.

UPTAKE OF FATTY ACIDS. Fatty acids are brought to the liver primarily as neutral fat from the intestine or the periphery. Sixty per cent of administered fat is utilized in the liver. Fat is transported from the peripheral depots to the liver when the nutritional supply of fat is inadequate to provide fuel, or when carbohydrate and amino acids are insufficiently available for bodily needs. This fat transport is under the influence of hormones, primarily those of the anterior pituitary. While the most important hormone is not established, corticotropin (ACTH) [1967] is at least as active in increasing liver fat as crude pituitary extract [258]. An adipokinetic factor different from ACTH but not separable from it by oxycel purification appears to be responsible [2818]. Pituitary growth hormone also rapidly mobilizes peripheral fat and causes its deposition in the liver, at the same time reducing the hepatic fatty acid dehydrogenase activity [1265, 1968]. The action of ACTH suggests that adrenal hormones released during stress are equally potent [1967]. Moreover, the fat-accumulating property of pituitary extracts, abolished after adrenalectomy, is restored by simultaneous cortisone administration [1968]. Fat accumulation in some types of liver injury may be the result of increased mobilization of fat from the depots under adrenal or pituitary stimulation.

STORAGE OF FATTY ACIDS. The liver stores fatty acids as neutral fat, as phospholipids, and, to a lesser extent, as cholesterol esters. Such fatty acid stores in the liver usually reflect the source of the fat by the degree of saturation [2572]. In starvation, liver fat resembles depot fat, whereas after feeding it approaches the composition of dietary fat. In the fatty liver the fatty acids increase, owing to accumulation of neutral fat and cholesterol esters rather than of phospholipids.

Neutral Fat

The normal human liver contains approximately 3.5 gm neutral fat, and the rat liver 4.5 to 5.0 gm per 100 gm wet tissue. This is the result of new formation, destruction, and deposition of fat absorbed from the intestine or mobilized from the periphery.

ABSORPTION FROM THE INTESTINE. The lipolytic theory of Verzar states that ingested neutral fat is completely split by lipases in the intestine into fatty acids and glycerol. The fatty acids are emulsified mainly by bile acids and also by phospholipids [23, 3338]. For passage through the intestinal wall fatty acids are probably incorporated into phospholipids [2572] or cholesterol [528], although evidence to the contrary has been presented [3713]. Most of the fatty acids are recombined with glycerol to form neutral fat in the intestinal wall, and enter the lymphatic vessels, which deliver them to the blood, which in turn brings them to the liver and fat depots. Some fatty acids remain as phospholipid or esterified cholesterol [337, 528].

In contrast to the lipolytic theory, the partition theory of Frazer states that neutral fat under the influence of lipase is only partially split to finely emulsified mono- or diglycerides. These glycerides are emulsified, with the aid of bile acids, to particle sizes, which permits their absorption into the blood capillaries. They are carried directly to the liver, which, in turn, controls the distribution of the fat.

The short-chain fatty acids have been shown to be largely transported by the portal blood stream from the site of absorption [1784, 2747]. Much evidence speaks against the partition theory as far as long-chain fatty acids are concerned. For instance, fat emulsified with Tweens can be visualized in lymph capillaries but not in blood capillaries [3434]. Also, ingested, tagged long-chain

fatty acids and various natural and synthetic neutral fats can be almost entirely recovered from the thoracic duct lymph in cannulated animals [313, 314, 2738].

NEUTRAL FAT IN SERUM AND BILE. Serum neutral fat, averaging 3.1 mEq per liter as fatty acids, is influenced relatively little, and then inconsistently, by food. An exaggerated lipemic reaction after oral or intravenous fat administration occasionally occurs. The milky appearance of the serum, caused by an increase in neutral fat, may be masked by the action of emulsifying lipids such as phospholipids [26]. Neutral fat is found in bile in concentrations up to 1:10,000.

Phospholipids

Phospholipids serve in the transport of fatty acids, in the stabilization of serum colloids and lipids [26], and in the oxidation of fatty acids [796]. They consist of a combination of fatty acids, many of them unsaturated, with glycerol, phosphoric acid, and a base such as choline in lecithin, ethanolamine, or inositol, in cephalins (Table 1). Phospholipids represent integral constituents of every cell of the body. In hepatic cells free of fat droplets, they are present mainly in the small and large granule fractions [12, 589]. The liver not only forms its own tissue phospholipids but is the main site of formation of the plasma phospholipids, as well as of their destruction (Fig. 12).

INTESTINAL ABSORPTION. Fatty acids are transformed to phospholipids, particularly lecithin, in

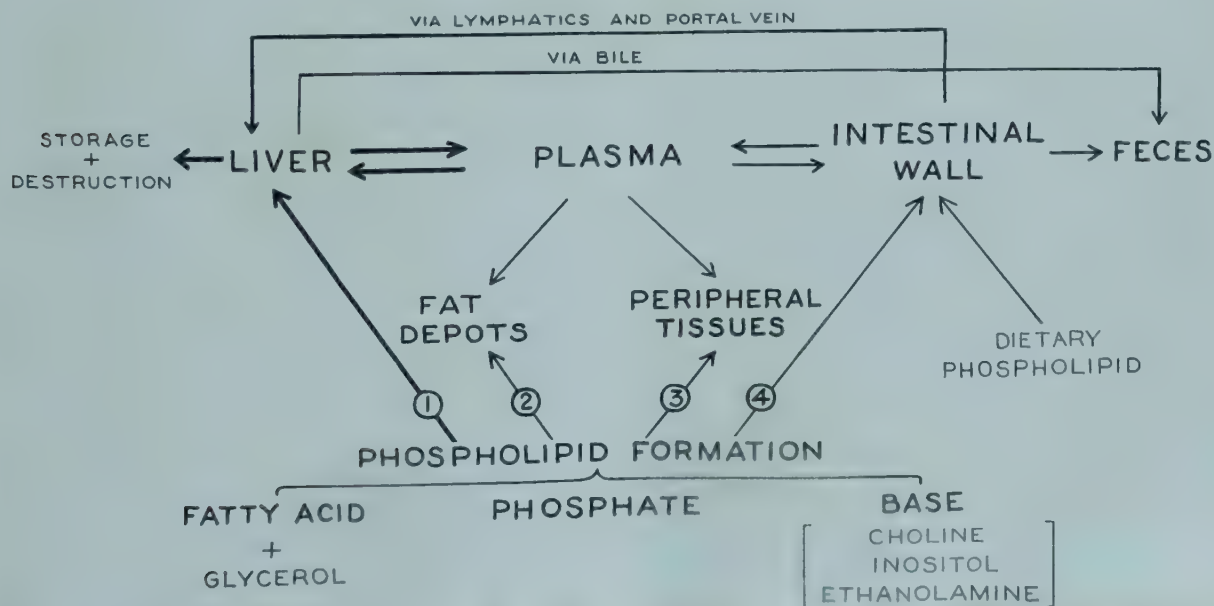


FIG. 12 Pathways of phospholipid metabolism.

the intestinal villi during absorption, while the fatty acids are discharged as neutral fats into the lymphatic vessels. Much of the phospholipid moiety remains in the intestinal wall. Nevertheless, some phospholipids are discharged intact into the lymphatic vessels in the absorptive phase [315] and are a source of plasma phospholipid. The intestinal lymph phospholipid increases two- or threefold in the postabsorptive state, even if no phospholipids are fed [338].

HEPATIC UPTAKE. The liver rapidly removes absorbed phospholipids from the blood stream and gradually releases them to other organs; in the liver the fatty acid component may be desaturated in preparation for use or storage in other tissues. The liver also takes up injected phospholipids; tagged phospholipids are found much longer in the blood of hepatectomized animals than in normal ones [941].

FORMATION OF HEPATIC PHOSPHOLIPIDS. The normal liver contains about 12 to 14 gm phospholipid per 100 gm tissue. The fatty acids in the phospholipids are mostly unsaturated. This may explain the preferential absorption of unsaturated fatty acids from the blood by the liver. Ingestion of cod-liver oil or linseed oil tagged with deuterium leads very rapidly to the accumulation of unsaturated fatty acids [3082] or to a large quantity of deuterium in the phospholipids of the liver. Studies with radioactive phosphorus [1022] and with tagged fatty acids [3545] indicate a very rapid turnover of phospholipids in the liver, exceeding that in any other organ. This has been interpreted either as formation in the liver for use in other organs or as increased breakdown by the liver [1474]. In the rat, the rate of synthesis of phospholipids is related to the activity of the enzyme choline oxidase [1371]. The turnover varies in different parts of the hepatic cell. It is slower in the nucleus than in the cytoplasm [1474]. It is most rapid in its small-granule fraction and less rapid in the large-granule fraction, which is believed to be the source of the plasma phospholipids [12]. Coenzyme A and ATP are needed for phospholipid synthesis. The rate of formation of phospholipids in the liver is retarded by the intake of cholesterol and by choline deficiency or experimental acute liver damage [2539], while it is accelerated by the administration of choline even *in vitro* [796], ethionine [2605], cystine [2567], or thyroxine [1035]. In experimental cirrhosis [337], in human acute hepatitis [521], and in both man and animals on

low-protein diets [664], the phospholipid turnover remains unchanged.

SERUM PHOSPHOLIPIDS. The serum-phospholipid level under normal circumstances is approximately 8 to 12 mg per 100 ml expressed as phosphorus, or 200 to 250 mg per 100 ml expressed as lecithin. Although the intestine contributes some of the plasma phospholipids, the liver represents the main source [338]. In general, the serum phospholipids reflect the balance of the phospholipid metabolism in the liver, especially the large-granule fraction. The plasma level tends to be lower in the presence of hepatic-cell damage and higher in biliary obstruction. However, the relative amount of the choline-containing phospholipid, lecithin, about 70 to 80 per cent of the total phospholipids normally, remains constant even in the presence of hepatic disease, when other lipid relations are altered [36]. Radioactive phosphorus studies indicate that plasma phospholipids are renewed at the rate of approximately 5 per cent per hour. This rate of renewal is related to the plasma-phospholipid level, in that the total activity of the plasma phospholipid at a given time after the injection of P^{32} is higher with high plasma-phospholipid levels, while the specific activity is not influenced [147]. These findings indicate that high plasma-phospholipid levels represent the result of increased formation rather than reduced destruction or utilization of the plasma phospholipids. Elevation is primarily encountered in conditions in which alterations of the ductules are found, suggesting that these structures are associated with serum-phospholipid formation or release. Intake of choline or methionine by normal people fails to raise the turnover rate [521], whereas in cirrhosis, where the rate is reduced, it may be accelerated by a single dose of 10 gm. Continued administration, however, is ineffective [521]. Cortisone or ACTH raises the plasma-phospholipid level [22], while adrenalectomy causes a sharp drop [3717].

BILE PHOSPHOLIPIDS. Hepatic bile contains small amounts of phospholipids, not exceeding one part in 10,000. The rate of formation is independent of the serum phospholipids, and elevation of serum phospholipids can not be explained by backflow of biliary phospholipids.

Cholesterol

Cholesterol is an alcohol derivative of a ring system (cyclopentanoperhydrophenanthrene). The ring nucleus itself is common to many biologic

substances (Fig. 21). Cholesterol occurs in the body as a free alcohol, as well as in the form of a fatty acid ester. The ubiquity of the free alcohol in the body indicates that it has important functions as a factor in cell permeability and as a means of absorption and transport of fatty acids.

SOURCES OF CHOLESTEROL. Cholesterol can be rapidly synthesized by the body from acetate [1386] and acetoacetate [376], acetone [347], pyruvate, butyrate, hexanoate and octanoate [376], and the isopropyl portion of isovaleric acid [3687] and is thus closely connected with the metabolic pool. It is probable that all the pathways of synthesis involve acetate, acetoacetate, acetone, isoprene, and squalene [3424]. Some of these pathways have been shown in man [1451]. Using tagged carbon compounds, synthesis has been demonstrated in the liver, intestinal mucosa, adrenal, testis, and skin [3164, 3303]. The rate of cholesterol synthesis by the liver would suffice for all the cholesterol requirements. In addition, the intestine absorbs cholesterol as such and transmits it to the lymphatic vessels, esterifying about 50 per cent in the process [528]. The presence of bile in the intestinal tract is essential for this absorption [3087], and most of the absorbed cholesterol is deposited in liver [1103] (Fig. 13). In most cells of the body cholesterol exists in a free form, and cholesterol esters are found only in blood, liver, and intestine.

HEPATIC CHOLESTEROL. The liver contains 0.25 gm total cholesterol per 100 gm fresh tissue (Table 1). Because of large amounts of esterifying enzymes, the bulk is in the form of cholesterol esters, with most of the fatty acids unsaturated.

The capacity of the liver to synthesize cholesterol depends on the nutritional status. Fasting or reduction of food intake [3344] and cholesterol feeding [1084] reduce hepatic cholesterol formation, while feeding carbohydrates and protein restores it to normal [3344]. Minimal degrees of hepatic damage reduce cholesterol synthesis from acetate, but additional damage increases the rate of synthesis, as does ligation of the common bile duct [1084]. Hepatic cholesterol formation is greatly reduced by hypophysectomy [3345].

The liver is the chief site of degradation of cholesterol. The turnover of labeled cholesterol in plasma stops when the liver is excluded from the circulation [1548]. Ingested cholesterol accumulates in the serum in Eck-fistula dogs, as well as in the liver, together with neutral fat [138]. Excess dietary cholesterol accumulates primarily in the liver; Kupffer cells seem to be the chief site of deposition and removal [1102]. The fat accumulation following cholesterol intake is dependent upon the fat intake. In fatty livers, the cholesterol content is increased proportionately more than the phospholipids. This increase is mainly the result of an excess of cholesterol esters. The percentage of cholesterol esters is especially high in the fatty livers produced by excessive cholesterol intake [258, 261], although this leads to a depression of the cholesterol synthesis in the liver [3303, 3346]. In fact, cholesterol has been considered the most potent stimulus for fat infiltration thus far discovered [2572]. Cholesterol competes with phospholipids for the unsaturated fatty acids in the liver, immobilizing large amounts of unsaturated fatty acids otherwise available for phospho-

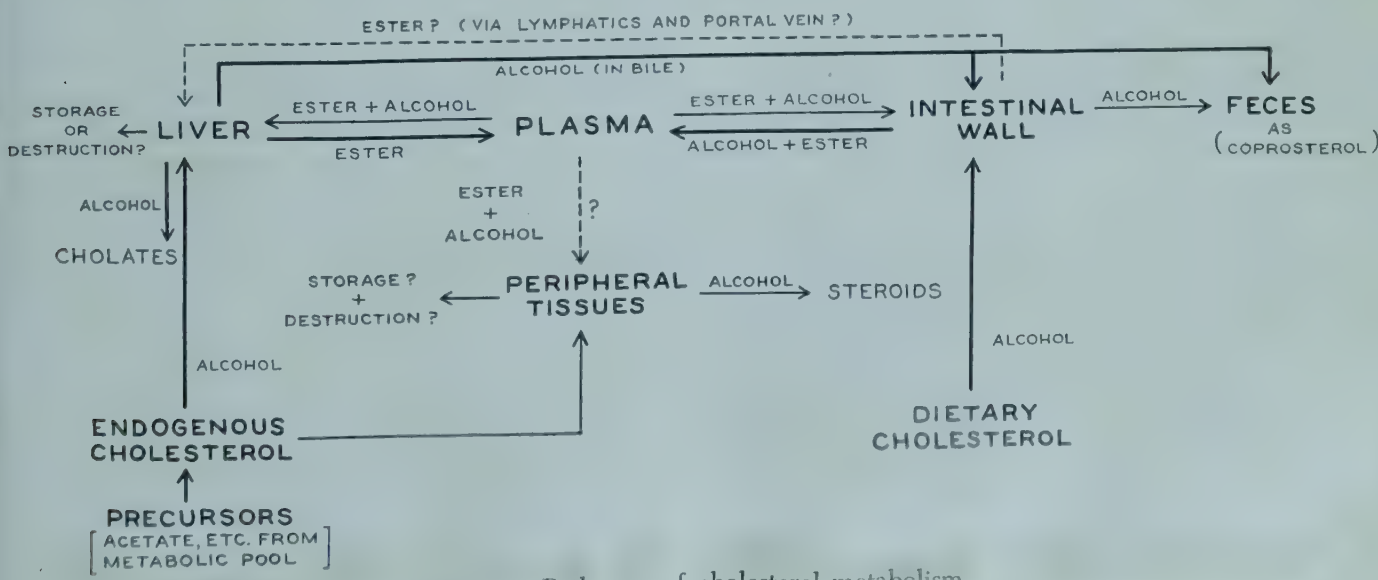


FIG. 13 Pathways of cholesterol metabolism.

lipid formation. This explains the reduced phospholipid turnover after cholesterol feeding and is probably the cause of fat accumulation, since cholesterol esters are apparently released more slowly to the blood. This mechanism is the basis of the antilipotropic effect of cholesterol, which is more pronounced in species with low serum-cholesterol levels, such as rabbits or guinea pigs. Large doses of choline, which remove fat more readily than cholesterol, cure the cholesterol fatty liver [1139].

The liver also contains cholesterol esterases, which may alter cholesterol metabolism under pathologic circumstances. The liver is the site of the most active breakdown of cholesterol. It oxidizes the side-chain carbon atoms to carbon dioxide and alters the sterol nucleus prior to excreting it in the bile as a saponifiable compound [2256].

SERUM CHOLESTEROL. Normally, serum cholesterol varies from 120 to 220 mg per 100 ml, of which 50 to 70 per cent is in the esterified form. The liver influences the level and the state of serum cholesterol. Some is derived from intestinal absorption, but most of it, as is the case with serum phospholipids, originates from the liver. Some of the serum cholesterol serves in fat transport to the tissues, and some tissue cholesterol, especially that of the adrenal, comes from plasma. The liver is probably the main site of cholesterol utilization, breakdown, and excretion, as it is of the phospholipids [1548, 2256].

INFLUENCE OF LIVER ON SERUM CHOLESTEROL. The liver influences the serum-cholesterol level in several ways (Fig. 13):

1. The formation of serum cholesterol is reduced in hepatic failure (see Serum Changes, Chap. 23).
2. Reduced excretion of cholesterol into the bile results in elevation of the serum level.
3. The liver regulates the level of the free cholesterol in serum by synthesis, destruction, and excretion [456, 2778].
4. The serum, like the liver, contains esterifying as well as hydrolyzing enzymes [3153, 3277], although cholesterol is mainly esterified in the liver before its release to the plasma [1386]. Thus, the ratio between esterified and free cholesterol is determined by the functional integrity of the liver.
5. Much of the serum cholesterol is protein-bound. Since the liver produces much of the serum proteins, it may influence the ability of the serum to carry cholesterol. The protein binding

is inferred because serum cholesterol is not ultrafiltrable and it migrates electrophoretically with alpha and beta globulins. Similarly, after ultracentrifugation, cholesterol is found with proteins and lipoproteins. Part is in the form of large molecular aggregates, altered primarily by ingestion of some foodstuffs and associated with atherosclerosis. These are increased in hepatic disorders with high cholesterol levels [2594]. Albumin has been claimed to stabilize cholesterol, and globulin tends to precipitate it, implying a further role of the liver [2616].

6. The serum-cholesterol level is dependent upon the presence of bile acids produced by the liver. The administration of cholates, or wetting agents, such as Triton, increases the serum-cholesterol level, presumably by altering the protein-binding ability of the serum [457, 1100, 1104]. The hypercholesteremia of nephrosis is also associated with hypercholatemia [2823], and this explanation suffices for the increased cholesterol levels frequently encountered in biliary obstruction [455].

7. Recently the relation between phospholipids and cholesterol in the serum has received much attention. The phospholipids, which are formed by the liver, act as stabilizing agents for cholesterol. In general, in conditions of hyperlipemia and liver damage, phospholipids increase proportionately more than cholesterol [2195].

The most important hepatic factors regulating the plasma-cholesterol level are thus hepatic synthesis, biliary excretion, and dietary intake, which are mutually interdependent. Factors possibly mediated through the liver include hormones [22], especially from the adrenal gland, heredity, age, and constitution.

BILIARY EXCRETION OF CHOLESTEROL. Relatively little unaltered cholesterol is excreted in bile, whereas large amounts of remnants of the cholesterol nucleus are excreted in the bile as saponifiable compounds which have been identified as bile acids [1548, 3088]. In human hepatic bile, the cholesterol content varies from 40 to 160 mg per 100 ml. In animals, it may be considerably lower; in rats and dogs, for instance, less than 14 mg per 100 ml is found. Almost all the cholesterol is free, and little is in the ester form. Its origin can be attributed to the hepatic cell, since the biliary excretion of cholesterol is considerably reduced in experimental hepatic damage and it continues to be excreted in isolated perfused livers [2778]. The cholesterol of the bile is generally independ-

ent of the blood level, but the lower serum level in hyperthyroidism is associated with, and possibly the result of, increased biliary excretion, while the reverse is true in hypothyroidism [2823]. The biliary cholesterol concentration in the bile does not depend upon the cholesterol content of the diet, and it continues to be excreted when the diet is free from cholesterol. Some cholesterol and cholesterol-breakdown products excreted in the bile can be reabsorbed from the intestine and thus participate in an enterohepatic circulation [3087].

Lipotropism

Substances which promote the removal of fat from the liver are called "lipotropic agents." Originally the term "lipotrope" was used to describe a substance which removes excess liver fat resulting from deficiency of choline or a related substance. In a wider sense it connotes a substance which removes excess fat regardless of its cause. The liver releases fatty acids mainly as phospholipids and cholesterol fatty acid esters, and possibly some as lipoprotein. Among the phospholipids, lecithin (phosphatidyl choline) is the most important. Since choline is necessary for the formation of lecithin, it has been considered a determining factor in fat removal from the liver and has thus been taken as the classic example of a lipotropic agent.

CHOLINE. Much of the understanding of fat removal from the liver centers around the functional and structural effects of choline deficiency. Choline may not only be important as a lipotrope, in the sense of a vehicle for fat, but may also be important in fatty acid oxidation in the liver [105]. Choline is provided in sufficient amounts by the average diet, and only under extreme conditions can choline deficiency become the determining factor in retaining fat in the liver. Choline $[\text{CH}_2\text{OHCH}_2\text{N}(\text{CH}_3)_3\text{OH}]$ (Fig. 14) requires three methyl groups for its formation, while the other constituents are easily available in the cell, as are the other constituents of the lecithin molecule. The availability of methyl groups seems to determine the rate of removal of fatty acids from the liver [868, 869]. The claim has been made that choline also increases hepatic circulation. The presence of the enzyme choline oxidase is necessary for the accumulation of fat. Animals without this enzyme in the liver, such as the guinea pig, do not develop fatty livers on deficient diets [1370]. The choline-deficient fatty liver was first recognized by the classic observation that fatty

livers occurred in pancreatectomized dogs [258]. On choline-deficient diets, fatty livers rapidly develop in young animals, in which growth increases metabolic needs. In addition, renal hemorrhages occur [258]. As the animal becomes older and the growth stimulus declines, fat decreases despite continuation of the diet [2041, 3699]. Choline requirements depend on age, sex, and strain [1345] and on the food intake, since fat disap-

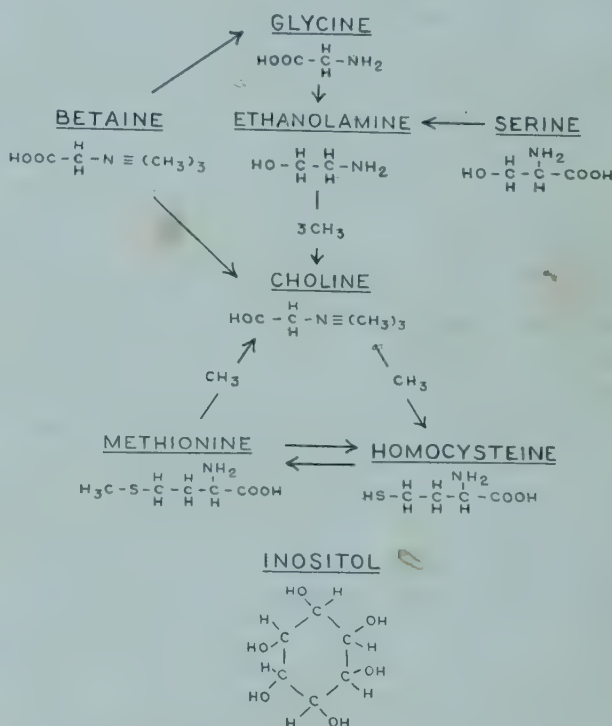


FIG. 14 Structural formulas and interrelationship of various lipotropic agents.

pears in starved choline-deficient animals [1370], although the ability to absorb dietary fat remains normal [3484]. Choline-deficient fatty liver develops best on protein-deficient diets not necessarily high in fat. An endocrine factor is involved as evidenced by the facts that fat deposition is more severe in male animals and that, in some circumstances, estrogens are lipotropic [929, 1281].

The addition of choline to slices of choline-deficient livers increases the synthesis of choline-containing phospholipids but has no effect on normal liver [796].

In rats on choline-deficient diets, slight bilirubin retention and bilirubinuria [1821], impaired Bromsulphalein clearance [3099], and disturbed bile formation [631] are noted.

Prolonged choline deficiency produces fatty cirrhosis in rats [1372, 1407] and in dogs [2902],

which may proceed to the formation of carcinomas [655].

LIPOTROPIC METHYL DONORS. Originally, methyl groups as precursors of choline were thought to be essential dietary constituents provided only by choline, betaine, and methionine (Fig. 14). Methionine transfers its methyl for the formation of choline, becoming homocysteine (transmethylation) [442, 868]. None of the other essential amino acids is lipotropic [878]. A pool of one-carbon atom moieties from many compounds, such as serine or glycine, now appears to serve as a source of methyl. These substances offer an additional source of choline. Vitamin B₁₂ and folic acid have been described as important cofactors in methylation or transmethylation [3249], explaining the lipotropic activity of vitamin B₁₂ under certain circumstances [1320]. However, the potent lipotropic factor present in crude-liver extracts and vitamin B₁₂ concentrates is not present in crystalline vitamin B₁₂ [2111].

Betaine, as a precursor of choline, can act as choline. Proteins are lipotropic methyl donors, since they provide not only methionine but also amino acids such as glycine or serine.

The supply of methyl groups present in the body is insufficient to provide all needs for choline in metabolic stress such as exists in growing animals, even if vitamin B₁₂ is present. Under such circumstances, methyl groups become essential dietary constituents. Methyl groups are required for other biosynthetic reactions. They are necessary for the formation of methionine, creatine, and *N*-methylnicotinamide. In the formation of creatine, glycocyamine is the nonmethylated precursor which is excreted in the urine in methyl deficiency [2484]. Methionine is a more readily available source of methyl for this methylation than choline, although choline mobilizes fat faster from the choline-deficient fatty liver.

OTHER LIPOTROPIC AGENTS. Constituents of phospholipids other than lecithin, such as ethanolamine [3213] and inositol [1138], also have lipotropic activity. This activity is less efficient than that of choline, since it is not noticeable on a high- or normal-fat diet [258, 261]. No evidence exists that inositol is an essential dietary constituent. Comparison of the response of various lipotropes is rather unsatisfactory and difficult [261]. Another factor which plays a role in fat accumulation or removal is niacin [2033], which prevents the fatty liver in rats on threonine-deficient diets [3083]. The fatty liver in threonine deficiency differs from

the one in choline deficiency. The threonine-deficient fatty liver is not prevented by choline and methionine [1385], and both phospholipid and nucleoprotein synthesis are depressed, while in choline deficiency, only phospholipid synthesis is decreased [477, 2125, 3083].

ANTILIPOTROPES. Compounds which deflect the lipotropic activity of choline by taking its methyl group, or which increase the demand for choline or other lipotropes, act as antilipotropic substances. Examples of the first group are nicotinamide and guanidoacetic acid, which utilizes the methyl for the formation of creatinine [3213]. The second group is exemplified by biotin, which increases the demand for inositol [1139], and by cystine and homocystine, which stimulate metabolism and promote growth [1281]. The effect of choline as the major lipotropic agent is occasionally obscured. For instance, choline given to animals on chronic ethionine or bromobenzene feeding increases the liver fat to amounts above normal, and thus choline administration can produce fatty liver. Fat deposition in the liver of rats on a low-protein-normal-choline diet increases when methionine is given because it creates a partial deficiency of threonine. In this sense, methionine is an antilipotrope under circumstances in which whole protein is lipotropic [1385].

PANCREATIC LIPOTROPES. The pancreas was formerly thought to form a hormone, called "lipocaic," necessary for fat removal. The lipotropic activity of pancreatic extracts has gained renewed attention following the observation that a pancreatic extract of low choline and methionine content reverses or prevents fatty liver of the pancreatectomized dog [351]. This substance is thought to act as a proteolytic enzyme which liberates methionine from protein [1330] and is ineffective in preventing fatty liver due to fasting or choline or methionine deficiency [351, 2747]. The fatty liver of pancreatectomized dogs may thus be the result of impaired intestinal absorption of protein and choline owing to the absence of pancreatic secretion.

OBJECTIONS TO PRESENT CONCEPT OF LIPOTROPISM. The concept of lipotropism is intriguing and important and was useful in the understanding of the mechanism and prevention of fatty liver. Several facts can not as yet be reconciled with it:

1. The methyl groups of choline are not essential for lipotropic action, since substitutes such as triethylcholine [2095] or arsenocholine have full lipotropic activity.

2. The lipotropic activity of methionine is not necessarily related to its ability to donate methyl groups for choline formation, and choline acts as a precursor of methionine in some instances (reversed methylation) [850].

3. In the fatty liver produced by choline deficiency and other means, the choline content of the liver is not reduced, and a normal or even increased phospholipid content has been found [361, 1345, 1610]. This has been denied by some [1024, 1040]. Nevertheless these findings do not support the view that lack of available phospholipids is the determining factor in the transport of fat to and from the liver or the oxidation of fatty acids in the liver [1610].

4. Whether nutritional choline deficiency ever exists in man has not been convincingly proved. The reduced turnover of phospholipids and its increase above normal after administration of choline or methionine to patients with fatty cirrhosis [521] suggest a relative choline deficiency under these circumstances. This is not found in

patients with nonfatty cirrhosis or with uncomplicated infectious hepatitis.

5. The accumulation of fat in some intoxications is scarcely, if at all, influenced by lipotropic substances. In most of these instances, abnormal transport of fat from the periphery to the liver can not be excluded. Nevertheless, disturbances of circulation or enzymatic activity possibly prevent the formation and utilization of the phospholipids and thus produce fatty livers (see Etiology of Fatty Metamorphosis, Chap. 26).

FATTY LIVER. Excess fat deposition in the liver results from a disturbance of one or more pathways of the movement of fat to and from the liver, viz., (1) deposition of dietary fat; (2) transfer of fat from fat depots; (3) transformation of dietary carbohydrate and protein into fat; (4) lipogenesis from the metabolic pool; (5) oxidation of fat in the liver; (6) release of fat to the depots. Each pathway is influenced by several factors (Fig. 15), and therefore the pathogenesis of fatty liver is complex.

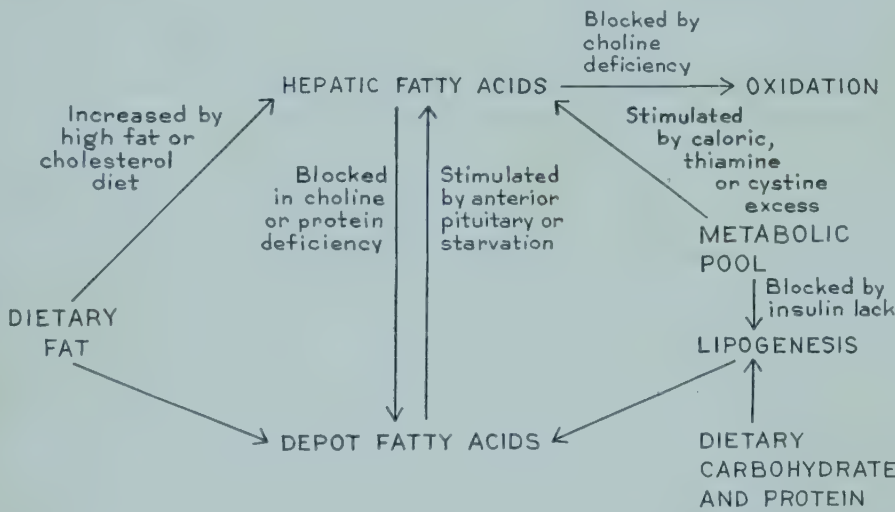


FIG. 15 Pathways of fat movement to and from the liver. In human fatty liver, several or all factors participate.

6

METABOLIC FUNCTION OF THE LIVER: PROTEIN METABOLISM

The liver is involved in almost all aspects of protein metabolism. In the anabolic phase, the formation of protein, the liver is important (1) in providing simple precursors for tissue-protein formation from the metabolic pool; (2) in the formation of its own protein; (3) as the main site of synthesis of the serum proteins; (4) in the storage of protein. In the catabolic phase, the liver breaks down protein to amino acids and deaminates them, processes which also occur elsewhere. The liver is the main site of urea formation from the nitrogen obtained by deamination. In

addition, other transformations of amino acids are preferentially located in the liver, and some phases of the breakdown of protein complexes, such as nucleoproteins, with uric acid formation, occur almost solely in the liver (Fig. 16).

Anabolism (Protein Synthesis)

Protein synthesis usually implies the linking of alpha amino acids by formation of peptide bonds. The enzymes responsible for this are as yet unknown, although their existence has been postulated (synthetases). The hepatic amino acids

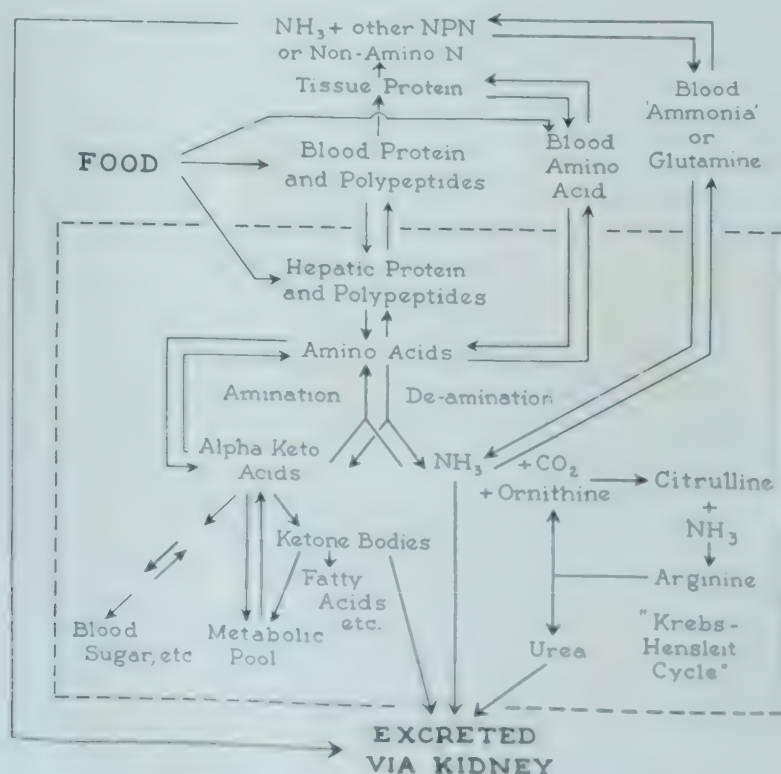


FIG. 16 Role of the liver in nitrogen metabolism. The area within the dotted lines represents those processes located within the liver.

(Table 2) are normally made available from three sources:

1. Amino acids absorbed from the intestine following hydrolysis of ingested protein. This proc-

Table 2 Amino Acid Composition of Beef Liver Corrected for Moisture and Ash and Calculated to 16% N

Serine.....	7.25
Arginine.....	6.63
Phenylalanine.....	6.06
Lysine.....	6.02
Threonine.....	4.79
Tyrosine.....	4.59
Histidine.....	1.98
Tryptophane.....	1.81
Methionine.....	2.90
Cysteine.....	1.12
Total sulfur.....	1.17
Organic sulfur.....	1.11

Source: Data from E. F. Beach, B. Munks, and A. Robinson: *J. Biol. Chem.* **148**:431, 1943.

ess has no relation to liver function provided that the intestinal enzymes are present in approximately normal amounts. The behavior of absorbed amino acids in portal vein blood has been studied after test meals [774]. In hepatic failure the formation of intestinal enzymes is disturbed, intestinal protein hydrolysis is consequently impaired, and amino acid release is depressed.

2. Reutilization of amino acids made available from endogenous protein breakdown.

3. Formation of amino acids from carbohydrates and fats via small carbon compounds of the metabolic pool. The nitrogen is incorporated into metabolites of fats and carbohydrates by such processes as amination or transamination (see Amination and Transamination, Chap. 4). Only some amino acids can be formed from nonamino acid compounds in and outside the metabolic pool. Others, also integral parts of the protein molecule, are formed in insufficient amounts, or not at all, and have to be provided either from endogenous breakdown of tissue protein or in the diet. Eight amino acids are essential in man (see Relations of Proteins to the Metabolic Pool, Chap. 4) [2809].

Hepatic Protein Formation. This has been discussed in the description of the morphologically visible protein granules (see Cytoplasm, Chap. 3) and of the cytoplasmic nucleoproteins (see Pen-

tose Nucleoproteins of the Cytoplasm, Chap. 3) which are important in this process. The concentration of hepatic protein, largely accounted for by the parenchymal cells, is usually expressed as hepatic nitrogen, since the hepatic nonprotein nitrogen represents only a very small fraction. The total hepatic nitrogen in man varies from 2.60 to 2.90 gm per 100 gm wet liver tissue, which represents about 17 gm protein per 100 gm liver [3595]. The hepatic nitrogen is influenced by the diet and by various endocrine as well as hereditary and maternal factors [1844, 2859]. In principle, the same types of protein have been identified in the liver as in the serum, namely albumin and several globulin fractions. The water-soluble proteins have been studied electrophoretically [906, 1183, 3138]. Most of them have mobilities comparable to those of the serum proteins. The fraction corresponding to alpha globulins is high, while albumin is quite low. The rate of formation of hepatic proteins seems to be similar to that of the serum proteins. The half-life of hepatic proteins has been given as 7 days [2953] and, using iodinated albumin, that of serum proteins has been given as 10 days [3162], although this last finding has been questioned [101]. Liver slices incubated with tagged carbon compounds showed a similar rapid rate of incorporation into hepatic albumin and serum albumin [3155].

Plasma Proteins. The plasma proteins are protective colloids and buffer substances. They maintain the blood volume and have an important role in blood clotting and transport of antibodies. The proteins in the blood stream are replenished at the rapid rate of 10 per cent per day [212, 3162], the globulin fraction being used up somewhat faster than albumin [212].

The liver is the main site of storage of the serum proteins. The hepatic cells synthesize the serum proteins, a function they share with mesenchymal elements, a large portion of which are also located within the liver. The liver synthesizes all plasma albumin and fibrinogen and much of the globulins [2295], although under normal circumstances most of the gamma globulin is formed outside the liver [3597].

Little newly formed protein appears in the blood stream in hepatectomized animals, and all plasma proteins drop, although tissue proteins are formed at a normal rate [3297]. Severe protein depletion [576, 922] or partial hepatectomy [253, 2782] results in a drop in plasma albumin; globulins, however, may increase.

In human hepatic disease associated with low protein levels, the incorporation of methionine labeled with radioactive sulfur occurs at a sub-normal rate [1769], while in nephrosis, with equally low serum-protein levels, the rate is higher than normal [1717].

The following plasma proteins, as separated by electrophoresis (Fig. 17), are assumed to be formed by the hepatic cells: albumin, alpha globulin, fibrinogen, and possibly beta globulin, in addition to other proteins recognized primarily by their biologic activity.

ALBUMIN. The liver is considered to be the sole source of albumin [2167]. The experimental evidence for the hepatic origin of albumin is derived partly from observations on dogs with Eck fistulas (portocaval anastomosis with ligation of the portal vein, diverting the portal blood from the liver), in which the level of serum albumin can not be maintained above edema levels [2167]. Similarly, after hepatectomy the albumin level drops [253, 2202] as the formation of albumin ceases [3297]. In dogs with albumin levels reduced by plasmapheresis, chloroform intoxication greatly retards repletion [2167]. Whether the hypoalbuminemia

which appears simultaneously with signs of hepatic-cell damage in protein-depleted dogs [922] is a result of liver damage or protein depletion is not clear. Also, liver slices form albumin [2574]. In human liver disease the serum-albumin level is reduced, even with high-protein feedings and a positive nitrogen balance [2661].

Human serum albumin is now thought not to be a homogeneous substance [1511, 2087], and two peaks are found after prolonged electrophoresis. In cirrhosis less than normal amounts of the faster component are present.

Serum albumin supposedly is replaced at a rate of about 7 per cent a day with a half-life of 10 to 17 days as measured with iodinated albumin [254, 3203]; this rate is the same in cirrhosis [905]. Studies using endogenous proteins tagged with sulfur suggest that the normal half-life is much longer [101].

ALPHA GLOBULIN. Alpha globulins, some of which are combined with lipids [546, 1883, 3699] and some with carbohydrates [2254], originate from the liver. They are separated electrophoretically into α_1 and α_2 fractions. Alpha globulin may be released in increased amounts under

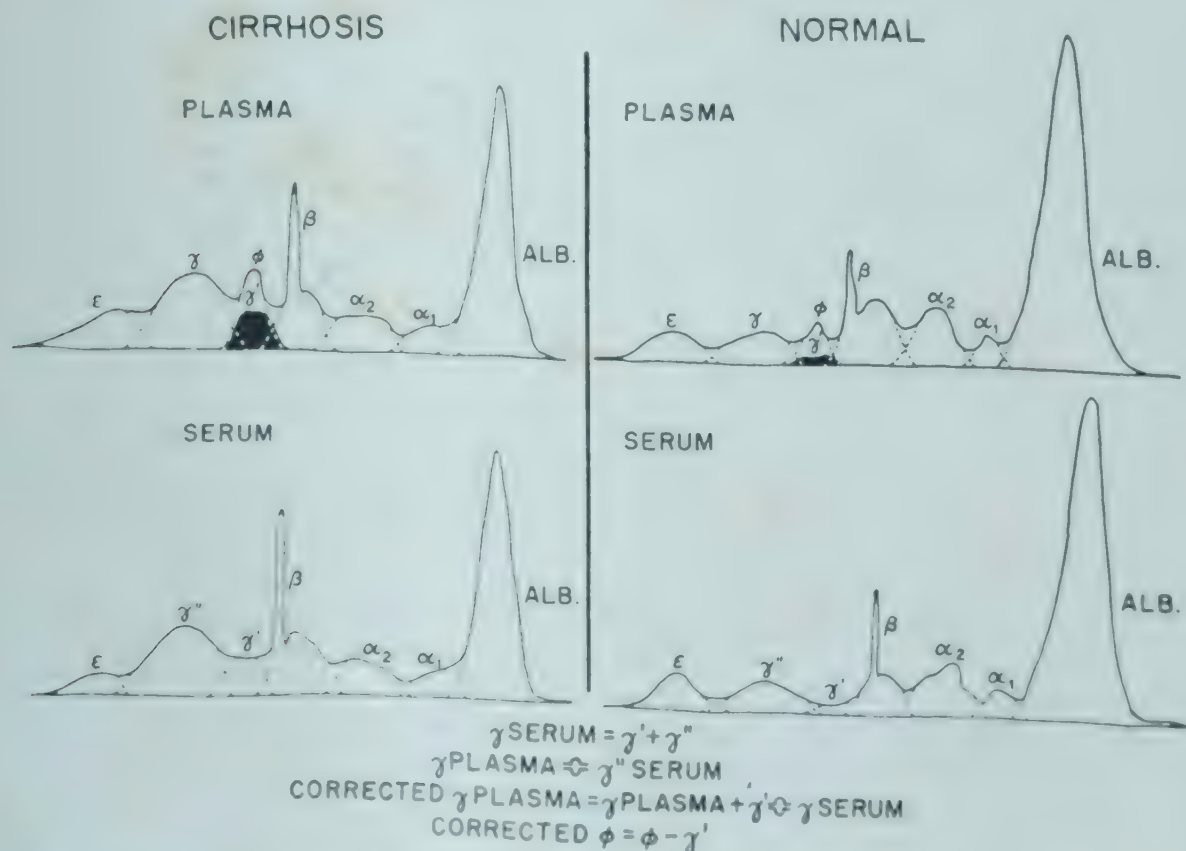


FIG. 17 Patterns of plasma and serum of a cirrhotic person and of a normal individual, with boundary electrophoresis, according to Tiselius. (Franklin, M., Bean, W., Paul, J., Routh, J., de la Huerga, J., and Popper, H.: *J.Clin.Investigation* 30:718, 1951.)

conditions of stress, when there is corticotropin stimulation, or in liver injury [2780, 2782]. The lipid-containing portion, chiefly in the α_1 fraction, disappears very rapidly in liver disease and apparently has a shorter half-life than albumin [1909]. In hepatectomized animals, this fraction fails to rise, but it does rise after partial hepatectomy; it returns to normal when liver regeneration begins [2379, 2782]. In human liver diseases a wide range of values is noted. In severe liver damage very low values are found, especially in fatalities after a protracted illness [2627]. In cirrhosis, the alpha globulin level is frequently below normal [2228]. In approximately 70 per cent of cases of extrahepatic obstructive jaundice values are above normal. Alpha globulin generally increases with a decrease in albumin [575, 1184], although this is not the case in hepatobiliary diseases [2627]. In these, the wide range is probably explained by two competing tendencies; namely, a tendency for the alpha globulin to rise with a drop in albumin, and a tendency for alpha globulin to drop owing to hepatic-cell damage. The level in the individual case is decided by the predominance of one or the other factor.

BETA GLOBULIN. The origin of beta globulin is not established as yet, and the factors governing its blood level are poorly understood. The iron-binding globulins and lipoproteins represent a large part of the beta globulins [1883, 2059]. In hyperlipemias, including those associated with hepatic diseases, this fraction increases [1883]. In obstructive jaundice it is elevated [2627], especially in protracted cases [2338] owing to increased lipoprotein. In parenchymal liver diseases, the values are generally higher than normal but may occasionally be very low [1260, 2228, 2627, 2766].

GAMMA GLOBULIN. These proteins are formed by the reticuloendothelial system and will be discussed later (see Globulin Formation, Chap. 14).

FIBRINOGEN. The formation of fibrinogen by the liver was established many years ago by experiments on frogs, which fail to produce fibrinogen after hepatectomy [2167]. More recently, fibrinogen has been found not to be regenerated in hepatectomized rabbits and dogs which have been bled and then transfused with defibrinated blood [2205]. This finding and the decrease of fibrinogen in severe hepatic insufficiency support the hypothesis that the hepatic cell is the site of fibrinogen synthesis. In man, reduction of fibrinogen occurs chiefly in fatal hepatic insufficiency [482].

Part of this reduction may be the result of excessive fibrinolysin. In various other forms of jaundice the fibrinogen level is normal, and in cirrhosis, despite the hepatic injury, it is often elevated to twice normal or more [1073]. The high electrophoretic fibrinogen peak is in part caused by gamma globulin, which has a similar motility (Fig. 17). Fibrinogen is increased in infection or after bone marrow irradiation, suggesting that part of this protein is formed in the reticuloendothelial system [2929]. Fibrinogenopenia in diseases not primarily hepatic has been repeatedly attributed to involvement of the liver [3663]. Hemorrhagic manifestations occur only with reduction of fibrinogen to below 30 per cent of normal, and fibrinogenopenia is of little importance in the hemorrhagic diathesis of liver disease.

PROTHROMBIN. Prothrombin, essential for blood coagulation, is formed solely in the liver, with the help of vitamin K. Recently, factor VII (see Factor VII below) has been separated from prothrombin. Many statements concerning prothrombin may refer to this factor. Hypoprothrombinemia not restored by vitamin K occurs in hepatic diseases [78, 335, 2082, 2610], in hepatectomized animals [2205], and in experimental animals intoxicated with carbon tetrachloride. Prothrombin is supposedly formed by the reticuloendothelial system [2929], but the evidence is unconvincing. Prothrombin is a glycoprotein with greater electrophoretic mobility than most other plasma proteins [2280]. It has a molecular weight of 140,000; whether it is a homogeneous substance or a composite protein [2060, 2682] is unknown. Vitamin K is not incorporated into the prothrombin molecule but probably serves as a prosthetic group of the enzyme which synthesizes prothrombin [2679]. Since 80 per cent of the prothrombin is used up in 24 hours, and since it can be fully restored in 4 hours, the serum-prothrombin level represents a balance between rapid utilization and production [507, 2679].

FACTOR VII. The stable factor VII, also called "convertin" or "serum-prothrombin conversion accelerator," acts on prothrombin in the initial steps of thrombin formation [1051, 2506]. It is apparently formed in the liver, as indicated by its rapid decrease in early parenchymal damage and in hepatitis. This drop is more pronounced than the decrease of prothrombin. It remains in serum on standing, while prothrombin decreases. Factor VII requires vitamin K for its formation, probably more than does prothrombin.

AC-GLOBULIN. AC-globulin acts as an accelerator in the conversion of prothrombin to thrombin [3485]. It is normally in an inactive state, plasma AC-globulin (factor V), and it is less soluble in ammonium sulfate and more sensitive to pH changes and heat than prothrombin [2984]. In contrast to prothrombin, it is also resistant to barium sulfate or aluminum hydroxide treatment [276]. Man has a rather low AC-globulin level compared with the dog or the rat, but has the highest prothrombin/AC-globulin ratio found in any species [2387]. AC-globulin is activated by the presence of a small amount of thrombin and is then called serum AC-globulin (factor VI) [2984]. Like prothrombin, it falls after hepatectomy [2205] and in experimental hepatic injury [2984]. It is reduced in human disorders only in severe hepatic insufficiency and in parahemophilia of Owren, and it is normal in biliary obstruction [1051, 2506].

GLOBIN. This protein, which makes up 95 per cent of the hemoglobin and which is a blood protein and not a plasma protein, is supposedly formed in the liver [3571].

SERUM ENZYMES. Many enzymes of the serum are formed by the liver. They are discussed elsewhere, since their protein moiety is not the most significant part of the molecule.

FERRITIN. This iron-containing protein with a high molecular weight serves in the transport and storage of iron (see Iron, under Mineral Metabolism, Chap. 8). It has recently been identified as the hepatic vasodepressor material (VDM) [2252], the vasodepressor activity depending upon sulfhydryl groups [2251] (see Vasoregulatory Functions, under Hepatorenal Relationships, Chap. 63). The iron-free protein portion, apoferritin, is also formed in the liver and has vasodepressor properties.

• **Regeneration of Plasma Proteins.** Rapid regeneration of plasma protein places a stress upon the liver which is created experimentally by plasmapheresis, the extensive and repeated withdrawal of blood with reinjection of the washed red cells. Animals so treated are in a chronic state of protein deficiency and can be used to test the efficiency of various substances in promoting the regeneration of plasma protein. Ingested as well as injected amino acid mixtures have proved efficient, provided that adequate amounts of the essential amino acids are present [2166].

Storage of Proteins. In addition to specific tissue proteins, the hepatic cells contain soluble

proteins with electrophoretic mobilities similar to those of serum proteins. This suggests that the liver, because of its size, plays a significant role not only in formation but also in storage of proteins.

Serum proteins may be subdivided into (1) those present in the blood; (2) the easily mobilized portion found within the tissue cells, particularly in the liver; (3) the small amount in the intercellular fluid. The protein of the latter two groups is available for release to the blood when the need arises (dynamic equilibrium) [3575], but the assumption of an equilibrium between tissue and plasma protein has been questioned [2]. The stored fraction of the serum proteins may represent as much as 90 per cent of the total available protein [922].

Catabolism

In the catabolism of proteins, the liver participates in several ways:

1. As in all other tissues, proteins are broken down to amino acids and polypeptides.
2. Together with kidney and brain, the liver deaminates amino acids. It is the only organ, in mammals, which forms urea.
3. It may also discharge amino acids into the blood (Fig. 16).

PROTEOLYSIS. Both tissue and serum proteins are broken down by various proteolytic enzymes, to low-molecular-weight peptides and amino acids. Proteolysis is increased as a result of cell damage, which leads to a greater concentration of non-protein nitrogen at the affected site, e.g., in the liver following anaphylactic shock [976].

DEAMINATION AND UREA FORMATION. The process of deamination and urea formation was discussed before as a common metabolic pathway (see Deamination, also Krebs-Hensleit Cycle, under Energy Provision, Chap. 4). At low blood-urea levels, the hepatic urea concentration is lower than that of blood urea, but at higher blood levels it rises and may even exceed that of the blood. The total NPN levels in liver and blood run fairly parallel. When protein formation is decreased as a result of cortisone administration, for example, excess amino acids are removed by deamination in the liver [587].

RELEASE OF AMINO ACIDS. In hepatocellular degeneration, amino acids are released to the blood, and values up to 25 mg per 100 ml of blood amino nitrogen are not uncommon in massive hepatic necrosis (normal levels being from 5 to 8 mg per

100 ml). This aminoacidemia and the associated aminoaciduria have been considered to be an expression of hepatic autolysis [1996]. Increased amino acid levels in the serum are of less practical clinical importance in liver disease than the demonstration of aminoaciduria, either by finding leucine or tyrosine crystals in the urinary sediment, or by various chemical, chromatographic, or microbiologic means. Such assays have shown that in liver disease urinary excretion of methionine, tyrosine, and valine is high, while that of lysine and histidine is low, and that of arginine, aspartic acid, and tryptophane is normal [863]. Paper chromatography indicates that other amino acids, such as cystine, serine, glutamine, and some peptides are often excreted in very large amounts, in addition to tyrosine and leucine [770]. Aminoaciduria is also noted in experimental injury caused by diets deficient in sulfur amino acids [1498]. In familial metabolic disorders with hepatic injury, such as Wilson's disease, aminoaciduria may occur without aminoacidemia [650] (see *Hepatolenticular Degeneration—Wilson's Disease*, Chap. 53).

CREATINE FORMATION. The liver forms creatine from arginine, glycine, and methionine in the presence of ATP and members of the Krebs cycle [305, 350, 2877]. Methionine or choline via methionine provides the necessary methyl groups [611, 868].

NUCLEOPROTEIN CATABOLISM. Nucleoproteins and nucleotides in the form of adenosine phosphates (ATP, ADP) and phosphopyridine nucleotides (TPN, DPN) are important in many enzyme systems as sources of energy. The nucleoproteins consist of a protein moiety and a highly polymer-

ized complex of nucleotides. Each nucleotide is composed of a purine or pyrimidine base, a pentose or desoxypentose, and phosphoric acid. The histochemistry of nucleoproteins has been discussed before (see *Pentose Nucleoproteins of the Cytoplasm*, Chap. 3). Extensive chemical studies of the nucleoproteins of the liver have been performed and much of the present knowledge of nucleoproteins is based on studies involving the liver, but few of the findings apply solely to this organ.

In the catabolism of the nucleoproteins, the protein moieties follow the same routes as other proteins. In man, the purine and pyrimidine bases are transformed to uric acid, which is excreted in the urine [3038]. The role of the liver in purine metabolism and uric acid formation is not fully known. In vitro studies have shown that rat liver homogenates are able to form uric acid from various nucleosides and nucleotides [2755]. Xanthine oxidases are necessary for this process, and inosine is a key intermediate. Enzymes oxidizing uric acid to allantoin have been found in animal livers [159] but are absent from the human liver. The liver forms some uric acid from smaller molecules in the metabolic pool.

The uric acid levels in the blood in hepatic disorders are not well established, and both elevated [409] and reduced levels in severe hepatic damage have been reported. Renal insufficiency may account for some of the elevation. More serum uric acid than normal is said to be protein-bound in hepatic disease [19], but this has not been confirmed. Uric acid is also excreted in the bile.

7

METABOLIC FUNCTION OF THE LIVER:
ENZYMES

The hepatic cell contains many enzymes, some of which can be demonstrated cytochemically. Others, measured only by their activity, are presumed to be present in the cytoplasm of the parenchymal cell.

These enzymes govern a great variety of chemical processes. As a matter of fact, all processes discussed in the preceding sections on carbohydrate, protein, and fat metabolism are enzymatic. Many enzymes, such as the various respiratory enzymes, do not occur solely in the liver but are found in most cells, while others are specifically located in the hepatic cell.

A chapter devoted to the enzymes is justified because of the diagnostic value of the serum activity of certain enzymes and also because of the interest in their morphologic distribution in hepatic cells.

Some of the enzymes circulating in the serum are mainly or solely of hepatic origin. The correlation between hepatic and serum enzymes is complex. In almost all types of hepatic injuries, the activity of most enzymes decreases, with the exception of alkaline phosphatase activity [2637]. In acute carbon tetrachloride intoxication in the rat, however, serum-esterase and xanthine oxidase activities increase, possibly owing to leakage from the liver. In chronic hepatic injury, reduced enzyme formation by the liver depresses serum-esterase activity [1109].

Since enzymes in serum as well as in liver tissue are proteins, their activity parallels variations of the hepatic proteins to some degree. Following protein-deficient diets and in complete starvation the enzyme activities drop parallel with or even more rapidly than the hepatic protein [2959]. Phosphatase is less reduced, probably because it is not found in the hepatic cells, whereas other

enzymes, such as arginase, rhodanase, cytochrome oxidase, succinoxidase, and xanthine oxidase, decrease much more rapidly than hepatic proteins [216, 2828]. In regeneration, the restoration of the enzymes is related to the rate at which the cells grow.

The serum enzymes primarily studied in the clinical evaluation of liver disease are alkaline phosphatase and the various esterases. Alkaline and acid phosphatases, esterases, and glucuronidases are those chiefly demonstrated histochemically. The chemical determinations in liver tissue of such enzymes as phosphatase, esterase, or arginase have been used to measure the functional activity of the liver.

Phosphatases

Alkaline phosphatase is found in all cells of the body. It transforms organically bound phosphoric acid esters to inorganic phosphates in an alkaline medium. It can also transfer phosphate from one compound to another [821]. Different enzymes are said to attack the different organic phosphorus compounds, such as nucleic acids, phospholipids, or phosphorus-containing carbohydrates. On the basis of histologic criteria, such differences, which have been repeatedly stressed [2228, 2436, 3272], do not play a great role *in vitro* [1218, 1887], but this does not exclude the possibility that specific phosphatases are active *in vivo* [410]. Two types of hepatic, serum, and intestinal alkaline phosphatase have been described: the larger fraction drops after fasting and rises after fat ingestion or protein depletion; the smaller one remains constant throughout [3369]. The latter is highly sensitive to cyanide and is not activated by magnesium, while the former behaves in the opposite fashion [2829]. Apparently, the phosphatases in various

organs are immunologically or chemically different, as are the phosphatases in different species [2928].

Hepatic Phosphatase. The localization of the phosphatase in the liver itself has been studied with the help of the method of Gomori or its modifications [1217, 2997]. Dry freezing of the specimen is necessary to obtain quantitatively reliable results, especially at low levels of activity [3482]. The validity of the cytologic demonstration of alkaline phosphatase has recently been questioned in view of possible post-mortem diffusion of the enzyme [2460]. Nevertheless, the method reveals useful information, and good correlation with chemical results of phosphatase determination has been reported, at least in man [3045]. In various abnormal conditions, alterations in the distribution of phosphatase have been described, but they are not of diagnostic value in liver biopsy specimens [596].

NORMAL DISTRIBUTION. The histologic distribution of alkaline phosphatase varies in different species [2436]. In most animals, including man, it is found in the bile canaliculi, ductules, and ducts, and occasionally in the sinusoids, whereas the hepatic cell itself is almost free of alkaline phosphatase [596, 1382, 3045, 3447]. Acid phosphatase, in contrast, is found in the cytoplasm of the parenchymal cell [2511, 3263, 3445]. Diurnal rhythmic variations in histologic distribution are seen; most of the alkaline phosphatase is found on the periphery of the lobule [1536]. Phosphatase in the nuclei is now considered to be an artefact [1217, 2460]. Certain specific phosphatases such as glucose-6-phosphatase are found only in parenchymal cells, concentrated about the nuclear membrane in the peripheral third of the lobule [572]. Adrenal cortical extract and androgens increase the hepatic phosphatase activity [1816, 1818].

BILIARY OBSTRUCTION. Under these circumstances increased activity of alkaline phosphatase is found in the bile canaliculi and sinusoids, and some is also found in necrotic parenchymal cells. Rupture of the bile canaliculi with regurgitation of phosphatase is thought to account for the increase of the enzyme in the sinusoids [1869], although this has not been accepted by all investigators.

HEPATIC DAMAGE. Alkaline phosphatase activity is found in the parenchymal cells [596, 3045, 3447], especially in necrotic cells in subacute yellow atrophy and cirrhosis [1869] and in the con-

nective tissue in subacute hepatitis and cirrhosis [3447]. It is increased in the hepatic tissue, as well as in the serum, in carbon tetrachloride intoxication [838, 1820]. In mice, increased alkaline phosphatase is seen in atrophic cells after fasting or in hydropic cells after protein depletion [3445]. In other experiments, the activity of hepatic phosphatase was found to be decreased, as was that of other enzymes, if the diet was deficient in protein [2294]; but if the liver was damaged by the diet, the phosphatase activity increased [1821]. In glycogen-storage disease, glucose-6-phosphatase is absent, although normal alkaline phosphatase is present [662] (see Glycogen-storage Disease, Chap. 53).

REGENERATION AND TUMORS. Following partial hepatectomy, the regenerating parenchymal cells are rich in alkaline phosphatase [2827, 3263], even with dietary protein depletion [2685]. Increased hepatic alkaline phosphatase accompanies the resynthesis of the basophilic material (PNA) in butter-yellow intoxication [2262]. In experimentally induced hepatic tumors, the tumor cells show variable alkaline phosphatase activity [1670, 3584], while much is found in the rapidly proliferating biliary epithelium [2552] and even in necrotic areas [1275].

Serum Alkaline Phosphatase. Serum alkaline phosphatase originates from the osteoblasts, from the intestine [1034, 2171, 2172], and possibly from the liver. Intestinal phosphatase is released into the lymphatic vessels during fat absorption and reaches the blood stream [1034, 3369]. Alkaline phosphatase is not excreted by the kidney in man, as it is in some animals [481, 1037], but is removed by the hepatic cells and excreted into the bile. This process has been confirmed by intravenous injections of phosphatase and by histologic demonstration of alkaline phosphatase in the bile canaliculi. The phosphatase supposedly moves through the hepatic cell bound to one of its substrates [1964]. Without being catabolized the enzyme is excreted into the bile constantly, indicating a maximal secretory rate. Its secretion is thus independent of the serum concentration of phosphatase as judged by its activity.

BILIARY OBSTRUCTION. The serum-alkaline phosphatase activity is increased in man [596, 1314, 1869, 3045, 3447] and in experimental animals [1382, 1869, 3447] with biliary obstruction. In the dog with complete biliary obstruction, phosphatase rapidly appears in the thoracic duct lymph, parallel with bilirubin, suggesting a simple

regurgitation of phosphatase from the bile ductules. In incomplete biliary obstruction, it rises far more than bilirubin [3387]. In some dogs with biliary fistulas, decreased biliary excretion coincides with a slight increase in serum-alkaline phosphatase activity, while in others greatly increased serum-phosphatase activity occurs with normal biliary excretion, indicating increased formation of the phosphatase under the experimental circumstances [835].

HEPATIC DAMAGE. In cirrhosis without jaundice and in primary and metastatic carcinoma of the liver [438], the serum-alkaline phosphatase activity may be great [2640]. It is slightly increased in hepatocellular diseases [2640], such as infectious hepatitis [2709]. Ligation of the hepatic vein in dogs increases the serum-alkaline phosphatase activity [2319].

ORIGIN OF EXCESS SERUM-ALKALINE PHOSPHATASE IN HEPATOBILIARY DISEASES. In bone disease, increased osteoblastic activity is the source of the increased serum activity, but in hepatic disease the source of the alkaline phosphatase is much more controversial. Two schools of thought attempt to explain the increased serum-alkaline phosphatase activity in hepatobiliary disease, particularly in biliary obstruction. According to one group, alkaline phosphatase of extrahepatic origin, chiefly osseous, accumulates in the blood or regurgitates from the bile canaliculi into the blood in biliary obstruction, while the moderate increase in hepatic-cell degeneration is attributed to minor interferences with biliary excretion [1314, 2334]. The other group claims that the increased serum-alkaline phosphatase activity in hepatobiliary diseases is hepatic in origin and that it results from increased hepatic production.

Evidence for Extrahepatic Origin of Excess Phosphatase. Simple interference with biliary excretion of phosphatase originating from bone is suggested by the fact that the increased serum-alkaline phosphatase activity in obstructive jaundice is inhibited by cyanide [1313] or oxalate. Bone phosphatase is similarly inhibited, whereas hepatic phosphatase is not. Furthermore, in hepatectomized animals, the serum-alkaline phosphatase activity increases [710, 1032, 2170]. The histologic distribution of phosphatase in biliary obstruction has been attributed by some to impaired excretion of extrahepatic phosphatase, rather than to increased formation in the hepatic cells [3045, 3447].

Evidence for Hepatic Origin of Excess Phosphatase. Considerable experimental evidence suggests increased alkaline phosphatase formation in the liver.

1. Transfusion of serum rich in alkaline phosphatase (produced by ligation of the common bile duct) results in prolonged increase in the serum-phosphatase activity without any increase in biliary excretion of phosphatase [482, 1089, 3480]. This suggests that the excess in extrahepatic biliary obstruction differs from the readily excreted intestinal or osseous phosphatase [1964].

2. The phosphatase activity increases much more following common duct ligation than after total hepatectomy [1088]. This speaks against impaired biliary clearance as the cause of the excess phosphatase.

3. Partial hepatectomy in dogs leads to parallel increase in serum- and hepatic-alkaline phosphatase activities [2492], suggesting that the phosphatase-rich, regenerating liver is the source of the increased serum phosphatase. This, however, has also been explained as a result of impaired elimination of serum phosphatase [2827].

4. The diet determines the degree of increase in serum-phosphatase activity following extrahepatic biliary obstruction [325, 1551].

5. In rats with ligated common ducts, the intestinal lymph contains less phosphatase than the serum, excluding the intestinal mucosa as a significant source of the excess phosphatase [1034]. In addition, removal of the intestine does not prevent the subsequent increase in alkaline phosphatase activity after ligation of the common bile duct [710].

6. The increased serum-alkaline phosphatase activity in dogs (but not in man) with hepatic damage is inhibited by cyanide, while that of the normal dog is not [834].

Possible Hepatic Site of Phosphatase Formation. Some histologic observations implicate the hepatic cells as the chief site. However, the ductules, which also contain phosphatase [441], may be the source of the large amounts of the enzyme in the serum in obstructive jaundice. In hepatic diseases, including obstructive jaundice [467], greatly increased serum-alkaline phosphatase activity is usually associated with increase of ductules. In individual cases this correlation between increase of ductules and serum-alkaline phosphatase activity is not always found. The functional

activity of the proliferating tissue may be depressed by severe hepatic failure.

The available evidence suggests that the increased activity of serum alkaline phosphatase in hepatobiliary disorders can be attributed both to hepatic formation and also, to a lesser degree, to piling up of phosphatase of extrahepatic origin. Hepatic phosphatase production appears to be stimulated more by cholestasis than by hepatic-cell damage (Table 3). Since alkaline phosphatase

Table 3 Activity of Serum Alkaline Phosphatase and Factors Operating upon It in Various Diseases

	Normal	Bone diseases	Extra-hepatic cholestasis	Parenchymal liver diseases	Hepatectomy
Hepatic production of phosphatase	Minimal	Minimal	Greatly increased	Increased	None
Clearance of extrahepatic phosphatase	Almost maximal	Maximal	Greatly reduced	Slightly reduced	None
Extrahepatic production of phosphatase	Normal	Greatly increased	Normal	Normal	Normal
Serum-alkaline phosphatase activity in Bodansky units	0-4 BU	Very high —>15 BU	Very high —>15 BU	Somewhat increased —4-15 BU	Slightly increased

activity depends upon the interplay of activators and inhibitors, increased activity may possibly be the result of deficiency of inhibitors or excess of activators rather than the result of increased amounts of the enzyme itself [482, 1964, 2436].

Esterases

These enzymes split carboxylic acid esters such as acetylcholine, neutral fat, phospholipids, and cholesterol esters. Several main groups, depending on the substrate and on specific inhibition or activation, are recognized, and several classifications can be used.

1. Ali-esterases preferentially attack non-nitrogen-containing fatty acid-alcohol esters [1218]. They are regularly found in the livers of most species, including man.

2. Pseudocholinesterase hydrolyzes preferentially choline esters of fatty acids longer than two-carbon atoms [3594] and also splits acetylcholine [123, 1218, 2998]. It is more sensitive to eserine and diethylfluorophosphate than is nervous system cholinesterase. It occurs in human serum and in liver.

3. Nervous system, or red cell, cholinesterase preferentially splits acetylcholine, which is active in the transmission of nervous impulses [123]. Red cell cholinesterase is altered in various anemias.

4. True lipase, chiefly formed by the pancreas, splits long- and short-chain fatty acid esters, including unsaturated ones. Its activity is enhanced by taurocholate and it is atoxyl resistant [1897, 2998]. The serum-lipase activity is not necessarily altered in liver disease unless the pancreas is involved.

5. Lecithinase, which splits phospholipids, is found in many organs, in pancreatic and intestinal juices, in bacteria, and in snake venoms.

6. Cholesterol esterase, which hydrolyzes cholesterol esters, is found in many sites, including the liver (in the microsomal fraction) [2955] and the serum [458, 2442]. Cholesterol-esterifying enzyme, found in the serum, is measured by the reduction in free cholesterol on incubation [3277] and is low in parenchymal hepatic disease [3376]. Bile salts are necessary for the activity of this enzyme [3276].

7. Vitamin A esterase is responsible for hydrolysis of vitamin A esters. This enzyme has not been clearly identified as yet.

Hepatic Esterases. The histochemical demonstration of the esterases in the liver depends upon the species as well as upon the substrate used [1218, 2402]. They have been demonstrated with Tweens or naphthol esters and subsequent azo dye treatment [2402]. In the rat, this method shows them to be mainly in the cytoplasm of the parenchymal cells, while Kupffer cells and bile duct epithelium are poorly stained.

Reduced esterase activity has been reported under abnormal circumstances, such as in fatty, precancerous, or cancerous hepatic cells [2218]. So far, the significance of the histologic esterase distribution is not appreciated, although the contrast to that of phosphatase is apparent. Similar results have been obtained by various chemical analyses [2402]. Hepatic cholinesterase activity is low in malnourished infants in the tropical zones [3500], and in experimental animals after carbon tetrachloride, butter-yellow, bromobenzene, and other intoxications [1109]. This reduction of the esterase concerns primarily the microsomal fraction [2487] and is apparently related to hepatocellular necrosis, since it does not occur in simple fatty metamorphosis [2020].

Serum Esterases. Serum esterase, whether determined with acetylcholine or other substrates, depends upon hepatic function. Its purpose is so far unknown. Reduction of serum-esterase activity in clinical hepatic failure has been repeatedly described [1212, 2922, 3444], although this is not specific; it also occurs in pregnancy and in patients with cancer [1970]. In experimental hepatic injury, the serum-esterase activity is also reduced [383, 964, 2094] but not after hepatectomy [1609]. In contrast to lipase or esterase of pancreatic origin, the esterase of hepatic origin is depressed by atoxyl or taurocholate [1897, 2998]. Acetylcholine esterase is not identical with another enzyme which, at least in rabbit serum, splits benzoyl choline [1971].

The protein of the serum esterase is related to [1970] and migrates electrophoretically with albumin [123], although some is also associated with the alpha globulin fraction [1214]. The esterase activity generally parallels the serum-albumin level [964, 1972], and determination of the serum-cholinesterase activity is considered a means of studying protein metabolism. After experimental destruction of esterase by di-isopropyl-fluorophosphate, its regeneration is depressed in liver damage [893, 1885, 3563], and successful therapy with albumin enables the liver to restore serum-esterase activity [1881].

Amylase

The liver contains only extremely minute amounts of amylase or diastase in comparison with the pancreas. These can easily be attributed to the pancreatic amylase present in the blood [1933]. Serum amylase is not derived from the liver, since the level of plasma amylase is not changed following hepatectomy [1032]. Under abnormal circumstances, amylase enters the hepatic cell, or amylase present in the hepatic cells becomes active. This may lead to hydrolytic glycogen breakdown via dextrine and maltose, after diphtheria toxin administration, for instance [3301]. The reported reduction of serum amylase in liver disease is not easily understood [1258]. It is not found in dogs made cirrhotic by repeated injections of carbon tetrachloride [1739].

Other Enzymes

Scattered and often unconnected observations have been reported concerning the relation of the liver to many other enzymes.

The activity of the proteolytic enzyme con-

cerned with the lysis of fibrin thrombi is increased in cirrhosis, and sometimes in other diseases [2717].

A hyaluronidase-inhibiting principle, probably protein, has been found in increased amounts in hepatic diseases, although it is significantly reduced in hepatic coma [3121].

Beta-glucuronidase is an enzyme related to the hydrolysis and formation of glucuronides in the detoxification process [1023]. It has been found in many tissues, especially in the liver, where it increases rapidly after administration of substances such as menthol, which have to be detoxified [1977]. This increase has been related to regeneration, although this relation has been questioned [2302]. Beta-glucuronidase has been demonstrated histochemically in the cytoplasm of the hepatic cells [1095] associated with the mitochondria [476].

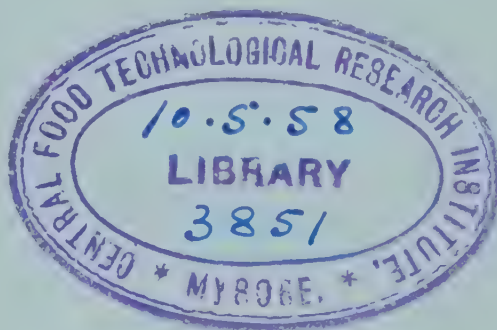
Other hepatic enzymes, not necessarily hydrolytic or oxidative in action, have been studied, especially with regard to tumor formation. For instance, a reduction of catalase or of arginase [1275] in tumor-bearing animals has been noted. Other influences on hepatic arginase, including those of fasting [2829], pregnancy [1043], and renal insufficiency [2401], have been reported. Depolymerases are increased in the precancerous state of the cirrhotic liver produced by azo dyes and are decreased with the appearance of the hepatoma [483]. Serum-tripeptidase activity is increased in liver disease and is more increased in biliary obstruction than in hepatitis or cirrhosis. It is also excreted in the bile, apparently behaving like alkaline phosphatase [1029]. The activity of betaine transmethylase, responsible for the methylation of homocysteine by betaine in the liver, depends on the amino acid composition of the diet and is reduced by vitamin B₁₂ or folic acid deficiency [799].

Serum-glutamic-oxalacetic transaminase activity increases 50 to 1,000 times normal in acute hepatocellular degeneration in man and in experimental animals [2327]. It also rises in extrahepatic cholestasis and in myocardial infarction, while in cirrhosis variable results are observed.

Many oxidative enzymes are found in the liver, some of them, especially cytochrome oxidase and succinoxidase, in the mitochondrial or large-granule fractions [2949]. Tyrosine oxidase is found only in the liver. Succinoxidase and cytochrome oxidase are reduced in nutritional deficiencies

[216], as well as xanthine oxidase [1866, 3565]. Hepatic xanthine oxidase has been related to susceptibility to cancer, since cancer-resistant mice have been found to have a more efficient mechanism for disposing of breakdown products of nucleoproteins [1006]. In hepatic damage, the

total activity of oxidizing enzymes is reduced, as can be demonstrated histochemically and chemically by the tetrazolium reaction. In rats intoxicated with carbon tetrachloride or bromobenzene, succinoxidase and amino acid oxidase are reduced [1109].



8

METABOLIC FUNCTION OF THE LIVER: VITAMINS, MINERALS, AND WATER

VITAMIN METABOLISM

The hepatic cell plays an important role in the metabolism of almost all vitamins, storing some and altering others. Changes in the vitamin metabolism affect hepatic function and structure.

Vitamin A

Vitamin A is an ionone ring with a long-chain unsaturated alcohol. In nature, it exists chiefly as the provitamin carotene, especially in plants. Upon hydrolysis this yields one or two, in the case of beta-carotene, molecules of vitamin A alcohol. The relationship between vitamin A and the liver has been extensively studied, since vitamin A can be not only reliably identified spectrophotometrically, but also localized histologically, by its green fluorescence, which fades rapidly [2625].

VITAMIN A TRANSPORT TO THE LIVER. Vitamin A of animal origin reaches the body as either free vitamin A alcohol or vitamin A ester combined with long-chain fatty acids. Vitamin A esters are split by pancreatic lipase in the intestine to yield vitamin A alcohol, which is absorbed through the intestinal epithelium. The alcohol is esterified in the villi to vitamin A ester, although some may be absorbed as such without hydrolysis. The vitamin A esters reach the blood stream via the lymphatic vessels [881], little, if any, going through the portal vein. Eventually they are taken up by the liver [592, 1509].

To pass through the intestinal mucosa, the fat-soluble vitamin A has to be emulsified, and for this process, bile acids are important. In hepatobiliary disorders the intestinal absorption of vitamin A is impaired, as shown by the low response of the plasma-vitamin A level to the intake of

large doses of vitamin A [2646, 2696]. This flat tolerance curve is the result not of increased avidity of the liver for vitamin A but rather of impaired intestinal absorption, since the tolerance curve (1) does not reflect the vitamin A content of the liver; (2) is not significantly altered by saturation of the liver by large amounts of vitamin A; (3) is flat in conditions with known impairment of absorption.

The impaired absorption may be caused either by a specific absorptive defect of the intestine or by alteration or reduction of the bile acids. The flat tolerance curve can be corrected by administration of vitamin A in emulsifying agents such as the Tweens and by bile salts [389], although not consistently [20, 2646].

When vitamin A is given to deficient rats, it appears in the Kupffer and the hepatic cells simultaneously [2625]. The uptake of vitamin A by the liver from the blood seems to be retarded in hepatic injury, since the serum-vitamin A ester level is elevated in such conditions [2642]. The administration of adrenal cortical hormone increases the hepatic vitamin A stores while depleting those in the kidney and lowering the serum level [326].

CAROTENE TRANSPORT TO THE LIVER. Carotenes, as well as biologically inactive carotenoids, chiefly of vegetable origin, are absorbed as such. Bile acids are even more essential for this than for vitamin A absorption [1262]. However, in hepatic disorders, with the biochemical methods used at present, the carotenoid levels are not necessarily low [2641].

Splitting of carotene to vitamin A, originally located in the liver, is now considered to occur mainly in the intestinal mucosa [557, 1192].

VITAMIN A IN THE LIVER. The liver stores about 95 per cent of the total vitamin A of the body [2341], chiefly in the ester form [592, 1192, 1257]. Fish liver is extremely rich in vitamin A [1698] in a cyclized form; in fresh-water fish, the liver contains chiefly vitamin A₂, which differs in its fluorescence from vitamin A [1269]. The toxicity to man of the livers of bears and seals is the result of their high vitamin A content [2792].

Biopsy and autopsy specimens of adult human livers contain, on an average, about 75 µg per 100 gm of vitamin A [2279, 2341, 2700]. Occasionally much more is found. In children the values are considerably lower [2341]; the content is minimal at birth and increases rapidly in the first months of life [920, 2625]. Livers of newborn rats are generally also almost devoid of vitamin A [1305], although relatively high levels have occasionally been encountered [1984, 2089]. Vitamin A is present in nuclei, mitochondria, and supernatant layers, the amount in each depending upon the amount present in the diet [2666]. In acute liver diseases, the concentration is not necessarily reduced [2012, 2625], whereas in cirrhosis it is reduced [1341, 2279, 2625, 2700]. Similarly, in acute experimental hepatic injury, vitamin A is not reduced [2643], whereas in chronic injury it is low and may be almost absent [1342]. In obstructive jaundice, low values are usually found [2279]. In nonhepatic diseases the vitamin A concentration is often reduced but, as a rule, not to the degree seen in severe liver disease. It is increased in nephrosis [1673] and sometimes in diabetes [2341, 2625].

The vitamin A concentration in the liver depends also on the dietary intake. Thus, in man, hepatic vitamin A concentration is reduced in malnutrition and increased after massive vitamin A therapy, although it is less increased in jaundiced persons than in normal ones [2625].

In experimental animals, the hepatic vitamin A content is an established criterion, not only for vitamin A nutrition, but also for the efficiency of vitamin A administration and for bioassay. Doses of vitamin A large enough to cause hypervitaminosis produce very high vitamin A concentrations [920]. The livers of vitamin A-deficient rats are free of vitamin A, and administration of vitamin A to these animals leads to its presence in the liver proportional to the amount given. Oral intake seems to be more efficient than parenteral administration [1946]. This has not been uniformly found [1922], perhaps because different forms of

vitamin A [3532], as well as carotene [1647], have been used. The menstruum in which vitamin A is administered is even more important in determining the hepatic concentration, aqueously dispersed vitamin A being more effective than oily solutions [2643, 3124]. Tocopherols appear important because vitamin E-deficient rats store vitamin A less effectively [2341]. Extensive studies on these covitamin relationships [1481] suggest that vitamin A destruction in the liver is inhibited during storage [2643] and that tocopherol possibly exerts an antioxidant effect. The hepatic storage of vitamin A seems to be independent of many biochemical conditions in the liver [594], such as choline deficiency [223, 594]. Administration of carcinogens reduces the vitamin A concentration of the liver [184, 504], especially in the mitochondria [5, 1197].

Histologically, vitamin A is recognized by a green fluorescence which rapidly fades during exposure to ultraviolet light (Fig. 18A). The fluorescence can be removed by lipid solvents [2625]. The specificity of the fluorescence for Vitamin A is indicated by its absence in vitamin A-deficient animals and its reappearance upon administration of vitamin A or carotene. No other substances restore this fluorescence. The amount of fluorescence is well correlated with the amount of vitamin A determined chemically [2625]. In the hepatic cells, vitamin A fluorescence is found in fine fat droplets arranged like a string of beads on the cell border adjacent to the sinusoids (Fig. 18B). Less bright fluorescence is seen in large fat droplets or in fine bars or droplets irregularly arranged throughout the cytoplasm. If lipofuscin gives a fat reaction, it also exhibits vitamin A fluorescence. There is, finally, a faint fading green fluorescence to be seen in parts of the cytoplasm owing to a small amount of dispersed vitamin A. The different sites of vitamin A fluorescence are responsible for the great variability of the fluorescent picture and are not necessarily dependent upon the lobular topography (Fig. 18C, D, E, F, G). In hypervitaminosis A, the bulk of the hepatic excess is found in the Kupffer cells, which are rich in fat [2451]. In the damaged liver, the vitamin A distribution may become very irregular in the individual cells and within the lobule [2625]. This irregularity found in acute hepatic-cell damage is not necessarily associated with chemically or histologically reduced vitamin A depots [2012]. The vitamin A content and fluorescence are reduced only with long-standing

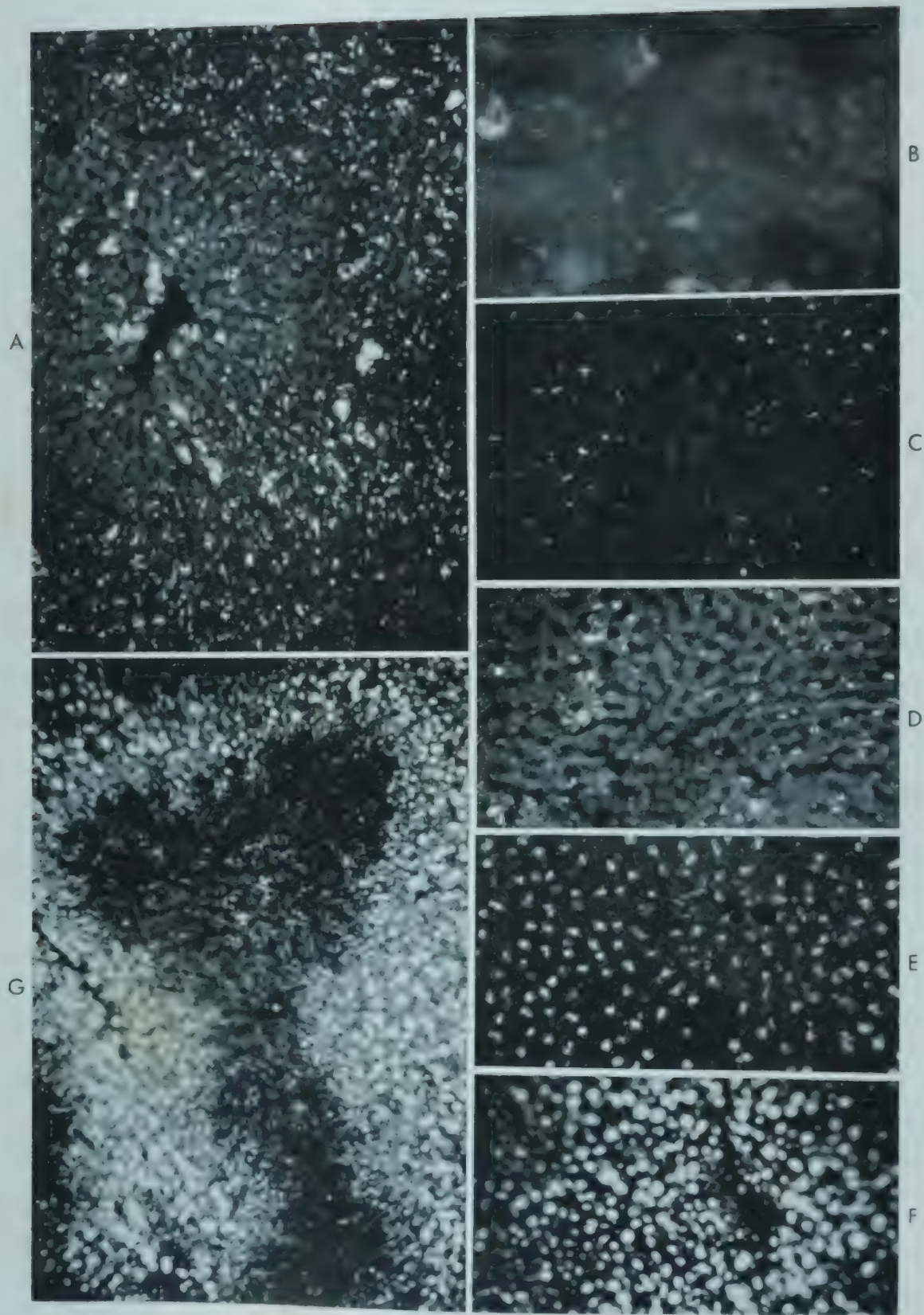


FIG. 15. Fluorescence photomicrographs of unstained frozen sections. A, human liver with normal vitamin A fluorescence in hepatic cells and in Kupfer cells, the latter being stronger. Fat droplets lining the epithelial cells and by large droplets in the Kupfer cells ($\times 340$). B, vitamin A fluorescence exhibited by small fat droplets A fluorescence imparted mainly by Kupfer cells (carcinoma of stomach) ($\times 115$). C, liver prior to vitamin A fluorescence of hepatic cells and Kupfer cells ($\times 115$). D, liver rich in of Kupfer cells, the hepatic cells being almost free (diabetes mellitus) ($\times 115$). E, strong vitamin A fluorescence fluorescence of fat droplets in nutritional fatty liver. The Kupfer cells do not impart fluorescence ($\times 115$). F, liver of rat intoxicated with carbon tetrachloride. Strong vitamin A fluorescence large fat droplets in the lobular center and of small fat droplets lining the hepatic cells in the periphery of the lobule ($\times 65$).

hepatic damage [2341, 2700], such damage probably resulting from impaired absorption of vitamin A from the intestinal tract.

CAROTENE IN THE LIVER. Carotene and carotenoids, the latter without biologic vitamin A activity, represent some of the pigments of the liver. Some carotenoids are associated with the mitochondria. The carotene concentration in the human liver varies between 0.2 and 3.9 mg per 100 gm [920, 1341, 2700]. In cirrhosis and other conditions with hepatic injury, the values are significantly lower [2700]. Carotene is not released by the liver to the blood stream.

RELEASE OF VITAMIN A FROM THE LIVER (PLASMA VITAMIN A). The liver normally regulates the plasma-vitamin A level; under abnormal circumstances this regulation is disturbed. Vitamin A is usually released as the free alcohol, in contrast to the esterified form in which it is transported to the liver. Upon administration of either alcohol [592] or epinephrine [3680], however, esterified vitamin A is released. Esterase activity has been postulated for the transformation of the vitamin A stores in the liver prior to release [1192]. In hepatic injury the release of vitamin A from the liver is impaired and the plasma-vitamin A alcohol concentration is greatly reduced [2625]. Although the plasma-vitamin A ester level may remain unchanged, the total vitamin A level is much reduced (endogenous hypovitaminemia) [2641]. In acute hepatitis, the plasma-vitamin A level drops in a few days, while the liver maintains its normal stores [2625]. In contrast, if volunteers are placed on a vitamin A-deficient diet, the plasma-vitamin A level drops only after several weeks, because the liver is able to release sufficient amounts to the blood [394, 3464]. This retarded release in hepatitis occurs without quantitative change in the vitamin A-hydrolyzing enzymes [2642].

Histologically, displacement of the vitamin A to abnormal sites within the hepatic cell is associated with decreased release of vitamin A to the blood, as evidenced by low plasma-vitamin A levels under these conditions [2625]. This retention of available stores has been demonstrated in carbon tetrachloride-intoxicated rats on vitamin A-deficient diets; their livers retain vitamin A longer, although in abnormal sites, than normal controls [2643]. During recovery from hepatic-cell damage, the plasma-vitamin A alcohol level rises to abnormal heights, apparently owing to excessive release as a result of the return of vitamin A to its normal histologic sites and possibly

owing to restitution of esterase activity [2643, 3181]. Disturbed vitamin A release by the liver can induce signs of vitamin A deficiency. Dark adaptation may become poor in cirrhosis before the liver is depleted of vitamin A [1341, 3641].

RELATION BETWEEN HEPATIC AND SERUM-VITAMIN A CONCENTRATIONS. Hepatic and plasma vitamin A in the rat are often parallel [1546, 1983], especially in early vitamin A deficiency [1859]. In depleted animals vitamin A can still be found in the blood and retina in the absence of hepatic stores [392]. Plasma and liver vitamin A are also parallel in infants [1984]. In adults, low plasma levels may be associated with a high or low hepatic vitamin A concentration [2640]. Apparently plasma-vitamin A levels parallel the hepatic concentration of vitamin A alcohol but not the total hepatic vitamin A stores [1192]. These complex relations make it impossible to decide whether the liver or the plasma vitamin A better reflects the state of vitamin A nutrition.

Vitamin D

The liver is the main site for the storage of vitamin D in the body. Fish livers are the richest natural sources of vitamin D. Little is known about the factors which influence the storage of vitamin D in man, and less is known about the influence of the liver on the metabolism of vitamin D than on that of vitamin A. Since bile acids are required for the absorption of vitamin D from the intestinal tract, disturbances of bile formation and flow result in faulty vitamin D absorption and also in faulty calcium absorption, with resulting osteoporosis and osteomalacia. This occurs in bile fistula dogs and in occasional instances of chronic liver disease [272]. Hypervitaminosis D produces calcification in the liver.

Vitamin E

The function of vitamin E, also known as alphatocopherol, a strong antioxidant, and its relation to the liver are not fully established. Vitamin E is stored chiefly in the liver [2235] and is excreted in the bile in a concentration equal to that in blood [2629]. The human liver contains from 0.4 to 3.4 mg vitamin E per 100 gm [2629]. The concentration is low in some hepatic diseases, because less is absorbed from the intestine [1791, 2629]. The plasma level, normally 0.15 to 1.5 mg per 100 ml [723], tends to be lower than normal in hepatic insufficiency, although not necessarily lower than in nonhepatic disorders [722, 1789,

1791, 2629]. In biliary obstruction and in some instances of cirrhosis with jaundice, the serum-vitamin E level is elevated [2629].

The curve of vitamin E concentration in serum after test doses of alpha-tocopherol is flattened in liver disease [1789, 2629] and is not elevated by saturation of the body with vitamin E [1791]. Nevertheless, the flat tolerance curve does not imply impaired ability of the intestine to absorb vitamin E in liver disease. Vitamin E is probably destroyed or altered in the intestinal tract in hepatic disorders [1785, 1791]. This, together with reduced intake, may explain the flat curve [1785].

Rats on low-protein diets deficient in vitamin E develop hepatic necrosis [1320, 1199, 1498, 1554, 2008] and are protected by vitamin E supplements [1319, 1320, 2969]. However, the cirrhosis which may develop on this low-protein diet is not prevented by adding vitamin E [1327]. Vitamin E deficiency also renders the liver more susceptible to carbon tetrachloride intoxication [1553]. These vitamin E effects are probably related to its antioxidation property. Vitamin E also prevents the deposition of fat pigments such as ceroid (see Ceroid, under Lipogenic-Lipotropic Imbalance: Experimental Fatty Liver Cirrhosis Syndrome, Chap. 50).

Vitamin K

Vitamin K is chemically a naturally occurring naphthoquinone derivative with a long side chain. It is fat-soluble but not water-soluble, and emulsifying bile acids facilitate its absorption. Vitamin K is necessary for the formation of prothrombin, factor VII, and possibly also factor V in the liver. The average diet provides sufficient vitamin K, and more is synthesized by intestinal bacteria [711]. About 0.5 μ g natural vitamin K₁ per kilogram of body weight daily is needed to maintain a normal prothrombin level in dogs. Sufficient vitamin K is absorbed from the diet if 5 ml bile per kilogram per day is present. Large doses of vitamin K₁ orally maintain the prothrombin level even in the absence of bile [2681].

Prothrombin deficiency with a hemorrhagic diathesis resulting solely from inadequate vitamin K in the diet is rare in man [1692, 3489]. Vitamin K deficiencies occur in various hepatobiliary disorders as a result of faulty bile secretion [1434, 2677]. In obstructive jaundice [2669] or hepatocellular insufficiency [1464], sufficient vitamin K may not be absorbed.

Even in the presence of vitamin K the damaged liver may be unable to form prothrombin, factor VII, or factor V (see Prothrombin Complex, under Clinical Manifestations of Hepatic Insufficiency, Chap. 23). The liver stores vitamin K [2067] but is unable to do so if damaged, which accounts for some endogenous prothrombin deficiencies and resulting bleeding tendencies.

Vitamin B Complex

Most of the known members of the vitamin B complex are constituents of respiratory enzymes and are therefore related to liver function. Many of them are specifically connected with the liver, and most of them are stored there. Deficiency of vitamin B complex promotes fatty metamorphosis even with subsequent cirrhosis, especially if the diet is kept low in protein [839, 1058, 2752]; not all the responsible factors are yet known [833].

THIAMINE. Thiamine pyrophosphate is part of the coenzyme lipoic acid, important in the conversion of pyruvate from lactate [3046] and in the formation of acetate. It is the cocarboxylase in decarboxylation processes and has also been associated with fat synthesis from the metabolic pool and with deamination of adenine nucleotides. It thus represents a coenzyme in the basic processes of the metabolic pool, many of which are solely or predominantly located in the liver. The phosphorylation and dephosphorylation of thiamine to the active pyrophosphate occurs mainly in the liver and the kidney [2471]. This explains low serum-thiamine pyrophosphate levels [1222, 2471, 3610] as well as the increased urinary thiamine excretion after administration of a test dose in hepatic disorders [349]. The increased urinary excretion in thyrotoxicosis is also attributed to the associated hepatic damage [3611]. Intestinal absorption of thiamine is depressed in liver disease, especially in extrahepatic biliary obstruction, possibly because of the accompanying achlorhydria [2122].

The complex influence of the liver on thiamine metabolism explains the disturbed thiamine utilization [3610] and the rather frequent occurrence of thiamine deficiency in liver disease even if the nutritional intake is not reduced. Cirrhosis is commonly associated with clinical features of thiamine deficiency such as polyneuritis or Wernicke's hemorrhagic polioencephalitis [1651]. In beriberi, severe edema of the liver and of the gallbladder bed has been noted [945]. This can result from

abnormal permeability of the sinusoidal wall or from the concomitant heart failure.

Some types of fatty liver regress with thiamine administration [1599]. Thiamine improves the hepatic dysfunction of experimental hyperthyroidism [837], possibly by protecting glycogen stores. Thiamine is necessary for the estrogen-inactivating function of the liver (see Effect of the Liver on Estrogens, Chap. 62); excess estrogen in thiamine deficiency supposedly causes uterine fibroids and breast lesions [285]. Thiamine administration, however, increases the hepatic fat accumulation on a choline-deficient diet by increasing food intake and thus raising the requirements for choline. Riboflavin and pyridoxine have a similar effect [1138].

RIBOFLAVIN. Riboflavin is phosphorylated and incorporated into nucleoproteins to become the basic constituent of many active respiratory enzymes (flavoproteins). Riboflavin is stored in the liver, as well as in the heart and kidney, as demonstrated chemically [563] and histologically by fluorescence microscopy [916]. During digestion and assimilation, the hepatic riboflavin content increases [3266]. The livers of dogs [2669] and of rats [1727] contain 16 to 25 μg riboflavin per gram of fresh tissue [124], most of which is in the large-granule fraction of the parenchymal cell [2669]. Experimental riboflavin deficiency increases hepatoma formation by azo dyes [2903] or choline deficiency [3074] and may cause fatty metamorphosis [124, 2665].

NIACIN. The liver is probably the storage site of niacin, since it contains more niacin than any other organ. Moreover, the liver is chiefly and perhaps entirely responsible for the methylation of niacinamide, the form active in the formation of coenzymes I and II. In niacin deficiency, coenzyme I activity is low mainly in the liver [720]. Niacin is associated with porphyrin metabolism [2693, 3156]. It prevents the fatty liver caused by threonine deficiency [3083]. Niacin is formed from tryptophane, not in the intestine, as originally thought [917], but almost entirely in the liver [1583, 1829]. This occurs in at least three steps. First, tryptophane is changed to kynurenine by tryptophane peroxidase. Kynurenine is broken down to anthranilic acid by kynureninase, for which pyridoxal phosphate (vitamin B_6) is necessary as a cofactor. Anthranilic acid in turn is converted to niacin [1813].

Clinical niacin deficiency (pellagra) is not necessarily associated with alterations in the liver.

Niacin seems to exert a vasodilating effect on the liver, possibly accounting for the rise of indirect bilirubin after its administration [3176].

PYRIDOXINE. Pyridoxine is phosphorylated to pyridoxal phosphate, the active form of the vitamin, largely by the liver. In mice and rat livers, the pyridoxine concentration averages 6 μg per gram of fresh tissue. It is concentrated in the large-granule fraction [2669]. The liver can transform tryptophane to niacin only in the presence of pyridoxine [2971]. Pyridoxine deficiency leads to fatty livers [1359] and to kynurenine excretion in the urine associated with decreased kynureninase activity in the liver [1813].

PANTOTHENIC ACID. Pantothenic acid is used by the liver and other tissues to form acetyl coenzyme A, the active acetate of the metabolic pool and an important factor in the Krebs tricarboxylic acid cycle [2459, 2483], in the metabolic transfer of other acyl groups such as acetoacetate, succinate, or fatty acids [2020, 3424], and in detoxification processes such as hippuric acid formation [544]. Most, if not all, pantothenate in tissues is in the form of coenzyme A, with the vitamin bound to adenosine pyrophosphate (ADP), which is in turn bound to a thiol ester, beta-mercaptoethylamine. In pantothenic acid-deficient rats, fat is not transported to the liver and the animals do not develop fatty livers on hypolipotropic diets [2345].

FOLIC ACID AND VITAMIN B_{12} . These substances represent parts of the liver factor active in pernicious anemia therapy and are also recognized as growth factors. As nuclear maturation factors, they are related to the formation of nucleic acids in general. In severely damaged livers the erythrocytic nuclear maturation principle is not necessarily diminished.

Folic acid is deposited in the liver and is related to the biosynthesis of purine bodies and nucleic acids [2465]. Hepatic damage may or may not affect this deposition. Folic acid influences such enzyme systems of the liver as xanthine oxidase [2942], and it is required in the oxidation of tyrosine [2794]. Folic acid and its antagonists significantly influence the enzymes of the normal liver [3604].

Vitamin B_{12} , first isolated from liver tissue [2581], is probably stored in the liver and plays an important role in the formation of hepatic nucleoprotein [3206]. The relations of vitamin B_{12} or folic acid to methionine and choline metabolism are not established [798]. A growing volume of

literature refers to their lipotropic activity, at least under certain circumstances [446, 833, 841, 1716, 3249]. This is probably related to the effect of vitamin B₁₂ on transmethylation. In this sense it appears to be choline-sparing [2903]. A protective effect has been ascribed to it in carbon tetrachloride intoxication [1555, 1826], but it does not prevent the massive hepatic necrosis due to deficient diets [1322]. Vitamin B₁₂ deficiency reduces the activity of the hepatic respiratory enzymes and increases xanthine oxidase activity [3607]. It also decreases the sulfhydryl content of the liver, decreasing chiefly glutathione [2731].

BIOTIN. Biotin is a yeast factor found in liver which acts probably as a coenzyme. Biotin administration produces a fatty liver which responds specifically to inositol. It is said to act by raising the requirements for inositol [1138]. The biotin effect has been used as an explanation for the fatty liver supposedly produced by liver extracts.

Vitamin C

Vitamin C, which is a component of reversible oxidation-reduction systems in the body, has no known specific relation to the liver. It participates in the oxidation of aromatic amino acids and is related to carbohydrate metabolism. It influences the formation and size of the Golgi appara-

tus and may affect the excretion of bile pigment [2385]. Its concentration in the liver is not necessarily decreased by various hepatotoxic agents [2886], and it may actually be increased by some. It is reduced, however, by necrogenic diets [2009]. Relatively little vitamin C is stored in the body; what there is may be found in the adrenal glands, skin, hair, liver, and small intestine. From 1.5 to 7.0 per cent of ingested ascorbic acid can be recovered in the liver [445]. The decrease of glycogen [272] and collagen [925] and increase of fat [2856] in the livers of guinea pigs on scorbutic diets can not be explained at present.

MINERAL METABOLISM

On merely a quantitative basis, the liver, as the largest organ in the body, has a nonspecific effect on mineral metabolism which becomes particularly apparent in hepatic diseases.

Sodium, Potassium, and Chloride. **NORMAL PHYSIOLOGY.** In man, the liver contains 6.0 to 12.0 mEq sodium per 100 gm [3595] and about 40 mEq chloride per liter of liver homogenate [3602] (Fig. 19). The livers of normal rats contain 7.1 mEq sodium and 32.4 mEq potassium per 100 gm [1130], on the average. After administration of radioactive potassium, the liver contains

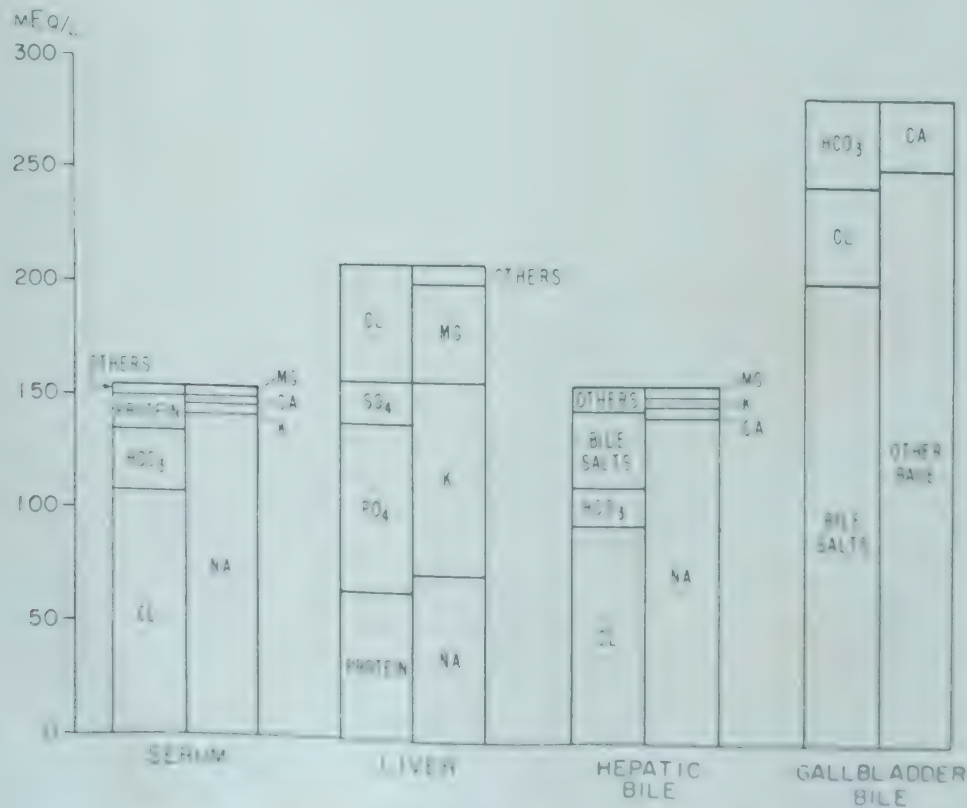


FIG. 19 Distribution of ions in serum, liver, hepatic bile, and gallbladder bile.

as much as all the muscles of the body (11 per cent) [2200]. The hepatic potassium concentration is influenced by alterations in the metabolism of carbohydrates; it increases during glycogenesis and decreases during glycogenolysis [994]. It also increases after shock or adrenalectomy [2200]. The glycogenetic action of insulin increases potassium in the liver, while the glycogenolytic action of epinephrine results in release of potassium to the blood [482]. Hyperpotassemia does not affect the hepatic potassium concentration but decreases the hepatic sodium [1130]. When hyperpotassemia is associated with a high sodium intake, coagulation necrosis of hepatocellular cytoplasm, with the presence of a few polymorphonuclear leukocytes, develops [479].

HEPATIC DISORDERS. Surprisingly little is known about the primary effects of hepatic disorders upon sodium and potassium metabolism. The main effect of the liver is nonspecific, as in any other parenchymal organ. Potassium is released from the parenchymal cells, whereas sodium enters them. In the anoxia induced by hemorrhage or by occluding the hepatic circulation, the hepatocellular sodium increases, while the potassium decreases [726, 3220]. In acute hepatic disorders serum-sodium and -chloride levels are slightly decreased [3131], with diminished sodium excretion and alteration of the urinary sodium-chloride ratio [945]. In addition, many hepatic disorders influence the serum-sodium and -potassium levels because of water retention in ascites and an indirect effect via the kidney. The serum-sodium level is lower [1530, 2426] and the potassium level higher [994] in cirrhosis with normal kidney function than in congestive failure [904, 980, 981, 2759]. Water retention also occurs after administration of sodium chloride [1001]. Because of the influence of nonspecific factors, including reduced dietary intake [64], the level of serum potassium, like that of other electrolytes, is low in hepatic failure. The effect of antidiuretic hormones and aldosterone is discussed later.

Calcium. In man the liver contains 6.0 to 10 mg calcium per 100 gm fresh tissue [3595]. More calcium is in the extracellular fluid (3.25 mEq per kg) than in the intracellular fluid (1.8 mEq per kg) [2200]. Calcium is bound to a liponucleoprotein complex [1906], and some is excreted into the bile. The low serum-calcium levels of liver disease [481] are caused by the reduced calcium absorption which accompanies the reduced fat

absorption, particularly in obstructive jaundice with the formation of insoluble calcium soaps. This has been associated with the hemorrhagic diathesis of liver disease, but the evidence is still not convincing [1603]. In addition serum calcium may be reduced in patients with severe chronic liver damage owing to malnutrition [64]. A protective effect of calcium in some forms of liver damage has been claimed [481].

Phosphorus. Phosphorus metabolism is related to the liver in several ways, especially through the role of phosphate in energy transfer and in carbohydrate metabolism. Radioactive phosphorus appears in the liver, first in compounds essential to the carbohydrate cycle, especially glucose-1-phosphate and ATP [1474, 2864], and subsequently in phospholipids and nucleoproteins. The microsomal fraction takes up relatively little radioactive phosphorus [1282]. The human liver contains 188 to 313 mg phosphorus per 100 gm fresh tissue [3595]. The rat liver contains about 30 mg, approximately 45 per cent in the form of phospholipids [1282]. In experimental carbon tetrachloride intoxication the acid-soluble phosphates in the liver increase parallel to the fat content, primarily owing to increased adenosine phosphates and phosphocreatine [940]. Characteristic alterations of the inorganic serum-phosphate level have not been found in hepatic diseases, although in severe liver damage it is reduced [64].

Iron. The liver, being the biggest iron depot in the body, plays an important role in iron metabolism (Fig. 20). It contains about 15 per cent of the body iron, while the serum contains 0.1 per cent [1247]. Alterations in iron metabolism result in characteristic alterations of the liver which will be discussed under iron-storage diseases. Body iron may be (1) bivalent heme iron in use in hemoglobin for oxygen transport or in cytochrome enzymes for intracellular oxygen transfer; (2) bivalent, soluble, inorganic iron in the upper gastrointestinal lumen and in transit across cell membranes; (3) trivalent iron bound to serum beta globulin for transport (siderophilin); (4) trivalent polymerized iron in depots [1247, 1945]. Iron is normally stored in the protein apoferritin as ferritin. This is apparently related to the antidiuretic pressure-regulating substance released from the liver (VDM) [3056]. Since the original demonstration of this substance, little progress has been made in its chemical identification [2251]. Excess storage occurs as a granular pigment, hemosiderin (see Siderosis, or Hemosiderosis, Chap. 53), in

which the same protein, apoferritin, is bound to larger amounts of iron [847, 1247]. Hemosiderin and ferritin are available for hemoglobin formation. Abnormal storage of iron may also be in the form of cytosiderin [1172] (see Iron Pigments, under Cytoplasmic Granules, Chap. 3, and Iron-storage Diseases, Chap. 53), in an inorganic form and in the vicinity of hemorrhages or in necrotic areas, in combination with other minerals, especially calcium, becoming unavailable for any purpose.

CONTENT IN THE LIVER. The iron content of the liver averages 19 mg per 100 gm in dogs [1008] and 28 mg per 100 gm in man [2908], representing from 0.01 to 0.05 per cent of its dry weight, or 0.5 gm. The iron content is greater in males than in females and increases with age, although it is maximal for a short period of time in the newborn. The liver contains heme iron, chiefly in the respiratory enzymes and some, loosely bound, in bile pigment (see Heme, under Nomenclature and Chemistry of Bile Pigments, Chap. 11). Normally 70 to 90 per cent of its iron is storage iron or ferritin [420, 846], making the liver the main site of iron storage in the body.

Under abnormal circumstances iron stores are increased to produce hemosiderosis or hemochromatosis [1688, 2375, 2967, 3171, 3667, 3669] (see Iron-storage Diseases, Chap. 53). The iron concentration is increased after parenteral iron administration when the concentration of iron is greater in the liver than in the spleen. After injection of radioactive iron, 10 to 35 per cent remains in the liver while 60 to 80 per cent is incorporated into the circulating red cells, again indicating the liver as the main iron depot [1007]. Most of the radioactive iron remaining in the liver is readily converted into ferritin [1339]. In some forms of experimental hepatic injury, the iron content is reduced [3414].

ABSORPTION AND EXCRETION OF IRON. The intestinal absorption of iron, mainly from the duodenum, is small in the adult, being 1.0 to 2.0 mg per day. Ferric iron is converted to ferrous iron in order to pass through the intestinal wall. It is then transported to the liver as siderophilin, which is normally 30 per cent saturated with iron, and stored as ferritin.

Since very little iron is normally excreted, only a minute fraction of the iron in the diet has been

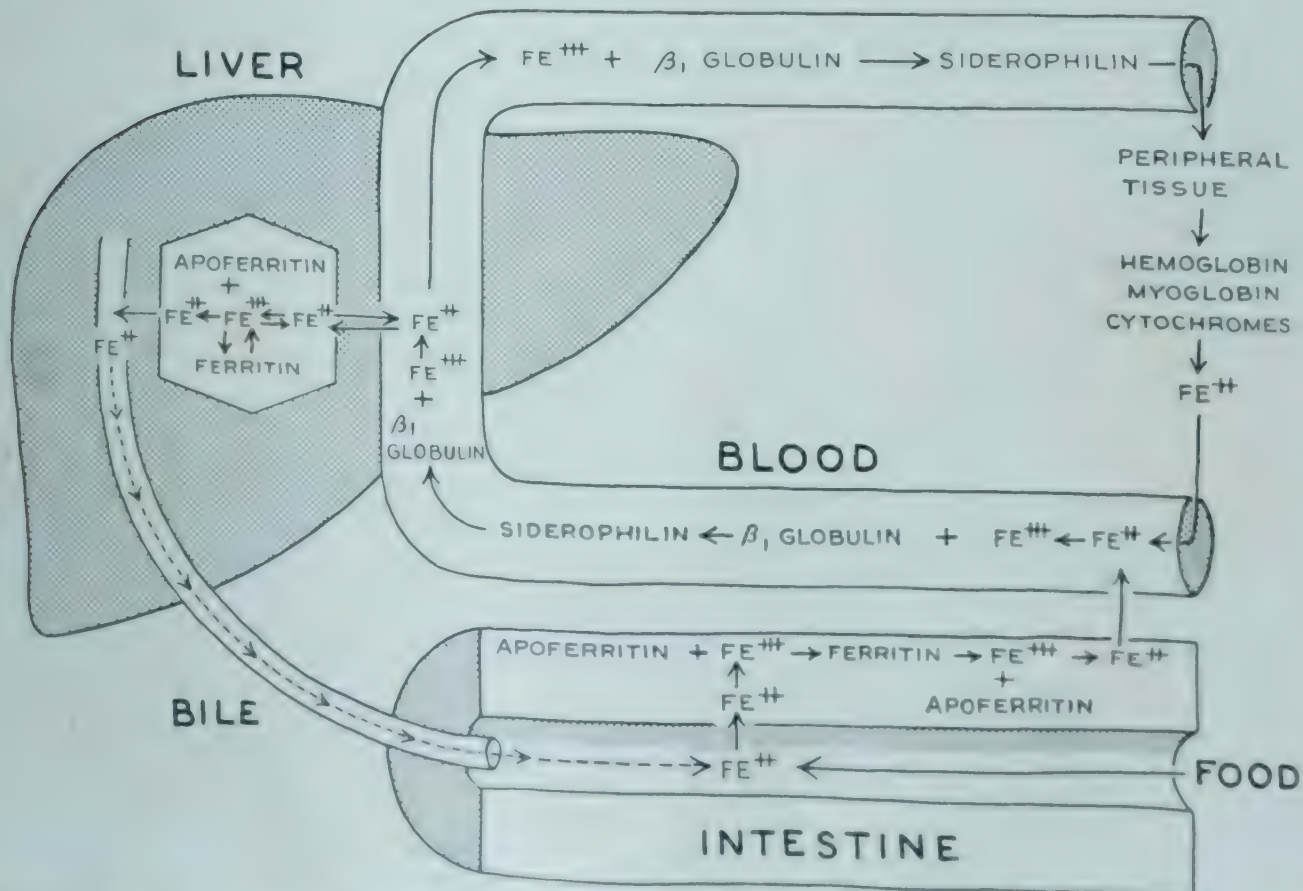


FIG. 20 Relation of the liver to the pathways of iron metabolism.

assumed to be absorbed. If the need for iron increases its absorption is increased [1335]. Iron absorption is regulated by protein components in the intestinal villi, apparently ferritin [1007, 1247], which temporarily store and then release the iron to the blood. This so-called "mucosal block" against iron absorption is surmounted [1007, 3222] in conditions increasing the needs of the body, such as childhood, pregnancy, and anemias. Then the additional iron absorbed is utilized at once and not deposited in the liver.

EXCESSIVE HEPATIC UPTAKE. If the mucosal block is overcome in the absence of increased demand, the iron saturation of siderophilin increases, and iron is stored in the liver either as ferritin or as hemosiderin, also found in Kupffer cells. This occurs in hemochromatosis (see Hemochromatosis, under Iron-storage Diseases, Chap. 53), in certain intoxications such as chronic copper poisoning [1172, 2947], in experimental dietary conditions [1765], and in human malnutrition [1486], especially in the Bantu [1172]. Pancreatic damage in malnutrition is associated with increased iron absorption, resulting in an excessive iron uptake by the liver [1172]. Excessive parenteral iron administration, for instance, by repeated blood transfusions or intravenous iron injections, also increases the hepatic iron stores. Faulty utilization of the iron in hemoglobin formation by the hematopoietic system, increased hemolysis, or infection may also result in increased hepatic uptake and storage of iron. Under all these conditions, which include all forms of anemia with the exception of iron-deficiency anemia, iron is stored at the expense of the circulating red cell mass [1008].

RELEASE OF IRON FROM THE LIVER. Tracer studies have indicated that some of the hepatic and hemoglobin iron can be reutilized [1007], although increased iron stores block the use of hemoglobin iron [1009]. Apparently, all nonheme iron in the liver is available, as shown by the removal of hepatic iron in dogs that are bled [1008]. In hemochromatosis the iron is also available [270]; even when iron deposits are so large that cell function is impaired, iron can still be mobilized [1008, 2577].

The relation of the liver to the serum-iron level is problematic, but some regulation has to be assumed. The excretion of iron into the bile is relatively small but increases, parallel to bilirubin, after hemolysis [1435]. If more iron is absorbed than the liver can store, the serum level rises, but it drops immediately if a low-iron diet is instituted.

Prolonged and excessive iron administration produces persistent elevation of the serum-iron level and saturates the iron-binding protein of the serum. By this time the liver has ceased to be a "shock organ" [1008] for iron absorption, and the uncontrolled serum-iron level results in iron deposition in extrahepatic sites, such as the skin.

In acute hepatitis the serum-iron level is elevated [2239, 2576], with a slight elevation of the serum-iron-binding capacity [391]. The elevation is explained neither by increased uptake from the intestinal tract, because the liver is low in iron, nor by faulty excretion into the bile, since it is unaffected in obstructive jaundice. Several other possibilities remain, such as a block to deposition in the liver, increased release from the liver, decreased utilization, or increased blood destruction [2576]. The elevation of serum iron lags behind the bilirubinemia in effervescent and defervescent stages.

Copper. Copper, small quantities of which are necessary for the formation of hemoglobin and respiratory enzymes [2791], is found in higher concentrations in the liver than in other tissues (Table 4). Normally the adult liver contains 20

Table 4 Inorganic Element Content of the Liver at Various Ages, in Milligrams per 100 Gm Dried Weight

Element	0-3 mo	3-12 mo	Over 1 year
Copper.....	32.49 ± 1.91	2.35	2.76 ± 0.10
Iron.....	89.84 ± 5.54	24.62 ± 7.14	47.66 ± 2.11
Lead.....	0.358 ± 0.027	0.457 ± 0.094	0.517 ± 0.019
Manganese.....	0.459 ± 0.026	0.608 ± 0.121	0.532 ± 0.015

Source: Data from G. C. Griffith, E. M. Butt, and J. Walker: *Ann.Int.Med.* 41:501, 1954.

to 30 mg per 100 gm fresh tissue, while 14 to 20 mg per 100 gm is found in children [2553]. The copper content of the liver is reduced in diabetes mellitus and primary or secondary hepatic neoplasms but is unaffected by other hepatic disorders. Excess dietary copper is stored in the liver [3730].

In the blood copper is bound either to albumin or to α_2 globulin in the form of ceruloplasmin (see Disturbance of Copper Metabolism, under Hepatolenticular Degeneration, Chap. 53). Serum copper is usually inversely proportional to the hemoglobin iron [1945], and this probably holds true for the liver, although in hepatitis serum-iron

and -copper levels are both elevated [391]. Copper is said to mobilize iron from the stores in the liver, but this point remains controversial. The alteration of copper metabolism in hepatolenticular degeneration (Wilson's disease) is discussed separately (see Chap. 53).

Trace Elements. Cobalt has recently been shown to be an integral component of vitamin B₁₂. Cobalt is stored in the liver [1267], but little is known of its metabolism.

Manganese, an activator of enzymes and related to protein synthesis, is present in the liver in concentrations greater than in any other tissue (Table 4) [1266].

Magnesium, an important factor in the Krebs tricarboxylic acid cycle, averages 15 mg per 100 gm fresh weight in human livers. The magnesium concentration in the intracellular fluid of the hepatic cell is about 30 mEq per kg in all animals tested, compared with 1.5 mEq per kg in the extracellular fluid [2200]. Serum-magnesium values are decreased in cirrhosis paralleling the severity of the disease [3310].

Hepatic zinc varies from 5 to 10 mg per 100 gm tissue [3595]. Under certain circumstances zinc may be lipotropic [2866].

WATER METABOLISM

WATER CONTENT OF THE HEPATIC CELL. As in other tissues, water constitutes a large portion of the hepatic cells. In the rat it accounts for about 70 per cent of the liver weight.

The liver contains the highest concentration of administered tagged water [3328]. The water content of the liver undergoes characteristic variations. In mouse livers, it decreases in the first 2 days of fasting and then increases to levels above the original values [2145]. Under normal circumstances the water storage is closely related to the glycogen content of the liver.

Much of the water is extracellular. Excessive increase of intracellular fluid is discussed under hydropic swelling.

OSMOTIC PRESSURE OF THE HEPATIC CELL. The liver tissue maintains an osmotic pressure greater than twice that of blood. It is isotonic with a 0.34

M sodium chloride solution, in contrast to erythrocytes, which are isotonic with a 0.15 M solution [2489]. The pressure is uniform under normal conditions but is often depressed by toxic agents, such as chloroform or carbon tetrachloride, by prolonged low-protein diets, and by obstruction of the common bile duct [2489, 2490]. Recovery from severe hepatic injury is usually accompanied by a return of the osmotic pressure to its former level [2490].

INFLUENCE OF THE LIVER ON WATER METABOLISM. The liver significantly influences water metabolism of the entire body, owing partly to the presence of hepatic vascular sphincters and partly to specific functions of the hepatic parenchyma. Abnormalities of fluid balance in liver disease are primarily the result of parenchymal dysfunction. Water is poorly excreted in liver disease, depending upon the type and severity of the disease [18]. Severe edema has been found in hepatectomized frogs, while rats kept on high-fat diets [3029] and dogs intoxicated with phosphorus or histamine [18] have abnormal water-tolerance test results. Oliguria is common during acute stages of hepatitis; diuresis heralds recovery [1654]. Plasma volume is increased in acute hepatitis, together with decreased plasma and urinary chlorides [1893]. Increased plasma volumes have sometimes been found in cirrhosis [2561], but low plasma volumes are as frequent [1584]. Increased water avidity of the skin occurs in clinical and experimental liver damage.

Hepatocellular dysfunction may either alter the formation of substances influencing water metabolism, such as the VDM-VEM mechanism [131, 1996, 3056], or result in inadequate neutralization of antidiuretic substances formed outside the liver [2592]. Disturbed water metabolism in liver disease is related to ascites formation (see Ascites, in Chap. 29, under Functional Changes in Cirrhosis).

HYDROGEN ION CONCENTRATION. The normal pH of the liver is 7.4 in the rat, slightly lower than that of the blood [1674]. In hepatomas the pH is considerably reduced and can be further lowered by glucose administration, which has no effect on the normal liver.

9

METABOLIC FUNCTION OF THE LIVER: BILE ACID METABOLISM

Bile acids are a specific metabolic product of the liver, probably of the parenchymal cells.

Chemistry of Bile Acids. Chemically, the bile acids are related to a group of substances with a similar ring structure, the cyclopentanophenanthrene nucleus, viz., cholesterol, steroid hormones, vitamin D, digitalis, toad poisons, and some carcinogenic hydrocarbons (Fig. 21). A variation in

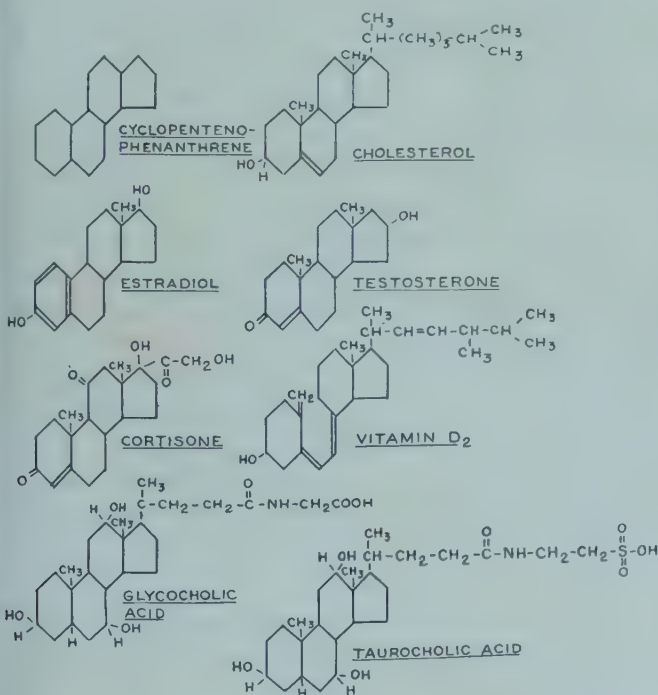


FIG. 21 Structural formulas of bile acids, cholesterol, and related steroids.

the steric configuration is the basic difference between the bile acids and the steroids; the bile acids are dextrorotatory. A terminal carboxyl group accounts for the acid character and also permits conjugation. In alkaline solutions such as bile, bile acids are sodium salts.

Variations in number and positions of the hydroxyl groups on the ring are responsible for the large number of different bile acids known [1411, 3125]. Difficulties in analytical methods have retarded chemical as well as metabolic investigations. Many natural, as well as synthetic, bile acids are known, and great species variations exist [1411, 3125]. In man, dog, and ox, the basic bile acid has three hydroxyl groups in the three, seven, and twelve positions and is called cholic acid or, chemically, 3-hydroxycholic acid [817]. It represents about 1 per cent of human hepatic bile [3125]. In some species two hydroxyl groups are predominant, viz., desoxycholic acid in the rabbit and chenodesoxycholic acid in the guinea pig [1411], differing in the positions of the hydroxyl groups. A monohydroxycholic acid, lithocholic acid, is found in man [3125], while in hog bile, dehydrocholic acid (hyodesoxycholic acid) is found [3126].

In man almost all bile acids are conjugated with either glycine or taurine, a derivative of cystine [817]. In lower vertebrates, they are conjugated with sulfates. In phylogenetically more advanced species, conjugation with taurine takes place; only in mammals are bile acids conjugated with glycine [1411]. In man, at least two-thirds of the bile acids is glycocholate, and one-third or less taurocholate. In dogs, taurocholic acid predominates [3125].

All cholic acid compounds, either conjugated or free, can be oxidized to ketocholic acids, which are also found in conjugated form in human bile. When nonoxidized bile is administered, some appears in human bile in the oxidized form.

Artificial products, especially those available commercially, are often oxidized. Dehydrocholic acid, for example, has an identical structural ar-

rangement as cholic acid except that the three hydroxyl groups are replaced by oxygen, the product being 3,7,12-triketocholelanic acid (Decholin, Ketochol).

All variations, in the sense of partial or complete conjugation and oxidation, exist in commercial preparations and probably in vivo. From the fragmentary knowledge available so far, it appears that three groups exist: (1) the nonoxidized hydrocholelanic acids, with various numbers and positions of the hydroxyl groups and conjugated in the naturally occurring form; (2) the oxidized conjugated form; (3) the oxidized nonconjugated form.

Formation of Bile Acids. The evidence that bile acids are formed solely by the liver is primarily circumstantial and is based on observations of the blood and bile concentrations of the bile acids after hepatectomy [339] or liver damage. In the hepatectomized dog, blood and urine are free of bile acids, while injected bile salts are completely recovered in the urine [339]. After hepatic intoxication or operative exclusion, the bile acid level drops significantly. In man, the relation of the bile acid level to hepatic diseases is far more complex [3045]. In the normal dog, 100 mg bile acids per kilogram is formed per day. This amount is reduced in hepatic infection or biliary obstruction and it is increased by administration of some amino acids or vitamin B₁₂ [2179]. Tracer studies have shown that, despite the stereochemical difference, bile salts can be synthesized from cholesterol [303, 3686]; this may represent the method of excretion of 75 to 85 per cent of the cholesterol [3088].

Conjugation of Bile Acids. The conjugation of bile acids is related to detoxification in general and is accomplished by peptide linkage with either glycine or taurine. It has been likened to hippuric acid formation and seems to be governed by the same factors [1664], i.e., it depends upon the supply of glycine or taurine and also upon an enzyme for the peptide linkage. In parenchymal-cell damage, the supply of glycine or taurine, as well as the enzyme, is reduced and is insufficient for the conjugation of endogenous as well as exogenous bile acids [74]. At least 80 per cent of the endogenous acids are conjugated under normal circumstances, but the percentage is less in hepatic damage [1664]. If small amounts of bile acids are administered, conjugation is almost complete. With larger amounts, a time lag is apparent, either for completion of the enzymatic action or for

mobilization of glycine or formation of taurine. This explains why nonconjugated bile acids are excreted initially under these circumstances [1664]. The glycine depots and the taurine precursors, such as methionine, cystine, or homocystine, can be exhausted. Administration of any of the precursors maintains taurocholate excretion. The human liver conjugates cholic acid more readily with taurine than with glycine, as shown by the increase of taurocholate over glycocholate after hepatic injury, such as temporary ligation of the common duct [1664].

Fate of Injected Bile Acids. The fate of injected bile acid depends on the form administered. If the natural, unoxidized, conjugated form is given, 90 per cent is recovered, of which 10 per cent is oxidized, and the percentage of keto acids is increased. If the oxidized form is injected, only 9 to 37 per cent is recovered quickly, although more may be excreted slowly [1664]. Injection of bile acid does not depress its formation.

Enterohepatic Circulation. Bile acids secreted in the bile are largely absorbed in the intestine, usually during lipid absorption. Bile acids separate from the lipids after passage through the intestinal wall and enter the portal circulation [1664], none normally entering the lymph. The absorbed bile acids are virtually all taken up by the liver and reexcreted in the bile, as evidenced by the great difference in cholate concentration between portal and jugular venous blood [1664]. Under normal circumstances, the enterohepatic circulation accounts for most of the bile acids secreted. Diversion through a bile fistula reduces the bile acid concentration of the bile, but this further stimulates the formation of bile acids. When the bile is fed to bile fistula animals, bile acid excretion is increased up to sevenfold, because of reabsorption rather than new formation [2934]. The liver is able to form at least ten times the normal amount if the bile is diverted. This increase gradually declines but returns significantly on feeding of meat or certain amino acids [600, 1436, 1749, 1827] (Fig. 22).

THE PHYSIOLOGIC LOSS OF BILE ACIDS. Although about 90 per cent of the bile acids found in the bile is retained by means of the enterohepatic circulation, small amounts are constantly lost normally and replaced by the synthesis of new bile acid. This loss is the result of three possible factors: renal excretion [1996], destruction by the liver [339], and, most important, fecal excretion [1664]. Virtually no bile acids normally pass into

the urine, but they do so after experimental removal of or damage to the liver [1664] and also in various hepatobiliary diseases [1996]. In biliary obstruction, urinary excretion takes the place of part of the fecal loss. Bile acid retention in general is the result of a disturbed balance between excretion and formation of bile acids [1664, 3045].

Abnormalities of Bile Acid Metabolism. The metabolism of bile acids can be disturbed by reduced formation in hepatic damage, reduced biliary excretion, or decreased hepatic uptake of absorbed bile acids.

In human acute hepatitis the blood-cholelate level is usually higher than normal, in contrast to the situation in experimental hepatic damage [1664, 3045], and it does not fall as the disease progresses. In biliary obstruction the blood level is higher than in hepatic-cell degeneration and decreases more rapidly, after relief of the obstruction, than bilirubin. In man, reduced formation is probably outweighed by reduced excretion and decreased hepatic uptake. In normal persons and in patients with biliary obstruction, after intravenous injection of cholate, the blood level rapidly returns to normal, while in parenchymatous liver diseases, the decrease is delayed [1664]. In hepatic-cell degeneration, therefore, the bile acids are not taken up by the parenchymal cells, in contrast to what happens in biliary obstruction, when they are taken up but are disposed of by means other than biliary excretion. The liver may temporarily store the bile acids to dispose of them gradually. The site of storage is probably the parenchymal cells and not the walls of blood vessels, as previously claimed. Bile acids may be discharged into the lymphatic vessels, a hepatobiliary-lymphatic circulation, which may explain

the high blood levels found in early biliary obstruction [2736]. In cholecystitis, absorption has been shown to occur through the gallbladder wall [817].

Function of Bile Acids. The defect in the intestinal absorption of lipids and fat-soluble vitamins in hepatic diseases can be at least partially alleviated by the administration of bile acids [389, 1262]. In the absorption of lipids and lipid-soluble material, seven processes are involved, all of which are influenced by the bile acids:

1. The surface tension of the various lipids is lowered by the bile acids, especially desoxycholates, bringing them into an aqueous emulsion. This process is important in fat absorption and also keeps cholesterol and fatty acids in solution in the acid gallbladder bile. It is also important for further fat digestion. Conjugated bile acids, in contrast to unconjugated ones, are acid soluble and, therefore, emulsify in the duodenum.

2. The pancreatic enzyme, lipase, which splits esters, in which form most lipids are ingested, is probably activated by bile acids.

3. The intestinal wall is penetrated by material in a water-miscible form. The production of water-miscible fatty acids results from the formation of easily dissociated compounds with bile salts, again primarily desoxycholates [3125]. These compounds pass through the intestinal epithelium, after which they are split, making the bile acids available for further absorptive activity after re-excretion. The actual mechanism of the hydro-tropic action of the bile acids is unsettled. The old choleic acid principle, entailing coating of the intestinal wall by bile acids to permit fat absorption, is still quoted. Bile acids are also necessary for the absorption of cholesterol [2952]. The an-

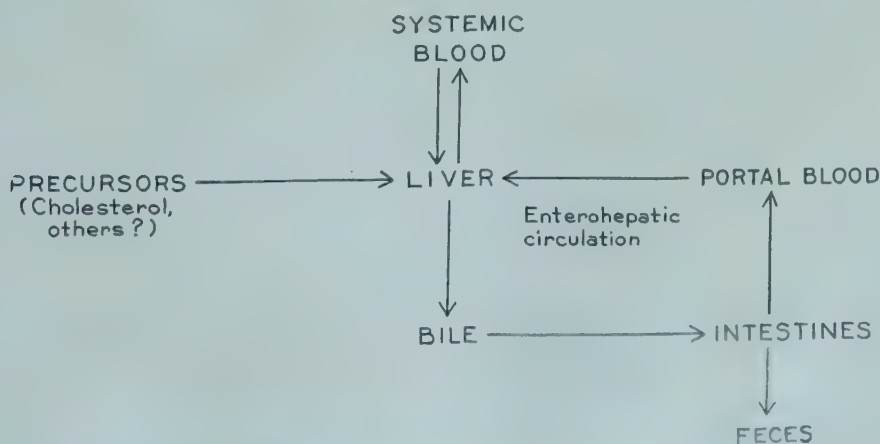


FIG. 22 Pathways of bile acid metabolism.

tirachitic action of bile salts is probably due to the improvement of vitamin D absorption. The effect on vitamin K is discussed under Hemorrhagic Diathesis in Chap. 23.

4. The absorption of substances other than lipids and fat-soluble vitamins A, D, E, and K is influenced by the bile acids. This includes the absorption of calcium, iron, and copper, which may form insoluble metal soaps with unabsorbed fatty acids in the absence of bile acids. Anemia develops in animals in which the bile flow is diverted, probably because of faulty absorption of an essential component. Bile acid administration relieves the condition. The absorption of some steroids, such as estrone and methyltestosterone, also requires the formation of water-miscible compounds [480]. Even water-insoluble alkaloids may be rendered absorbable in the presence of bile acids.

5. Bile acids influence the intestinal flora by bacteriostatic action. In the absence of bile acids, putrefaction of the stool is increased.

6. The water-retaining power of the bile salts has been held responsible for the hydration of the feces [1137]. This, plus a possibly specific effect upon gastrointestinal motility, imparts a laxative effect to the bile acids.

7. Bile salts are cholagogues which stimulate the flow of bile and increase the efficiency of its production. This essential function is discussed under Bile Formation, Chap. 12.

Toxicity of Bile Acids. The toxicity of the bile acids depends on their chemical structure. The most toxic is desoxycholic acid, followed in decreasing order by cholic acid, taurocholic acid, glycocholic acid, and dehydrocholic acid. Of the common conjugated forms, taurocholic acid is more toxic than glycocholic, although taurine and glycine are not toxic [1543]. The bile acids are the main toxic factor in whole bile, although whole

bile is more toxic than any of its constituents [1543, 3125]. The toxicity of the bile acids varies in different species, and different substances act as antagonists to the various toxic actions. Whole serum, owing possibly to its protein content, almost entirely neutralizes the toxic actions of bile acids. The specific toxic effects can be enumerated as follows:

1. Inactivation of cholinesterase by a physostigmine-like effect accounts for the bradycardia, the increased sweating, and increased neuromuscular activity by failing to interrupt transmission of stimuli. Administration of very high doses of bile acids may lead to muscular paralysis, probably owing to suppression at the myoneural junction. Phospholipids and atropine counteract some of these effects.

2. A specific hemolytic effect upon the red cells is inhibited by sucrose and lipids. The hemolytic effect is exhibited best in the test tube. It is difficult to demonstrate in vivo in the presence of nonhemolytic jaundice, in which the tendency for hemolysis is reduced rather than increased [1290].

3. An anticoagulant effect by binding calcium appears to be inhibited by glucose [1543].

4. A metabolic effect is caused by the need of cystine for the formation of taurine. Large amounts of cholic acid may drain the sulfur amino acids and thus produce toxic effects. Whether this plays a role in vivo is questionable.

5. Bile acids produce gastric ulcers if brought in contact with gastric mucosa. They may affect the renal epithelium (see Biliary Nephrosis, Chap. 63). The bile salts probably also have an effect upon the wall of the gallbladder. Cholesterol, lecithin, fats, and proteins act as protecting substances. Mucin, secreted by the lining of the bile ducts and gallbladder, also provides a protective covering and chemically neutralizes the bile acids.

METABOLIC FUNCTION OF THE LIVER: TRANSFORMATIONS ("DETOXIFICATION")

Many chemical reactions that take place in the liver are usually discussed under detoxification, since they represent the metabolic pathways by which endogenous or exogenous harmful substances are made innocuous. The end products of these processes are not necessarily less toxic than the original ones but may be more soluble and readily excreted in the urine. Similar pathways are used for transformation of nontoxic substances. Mitochondria of hepatic cells are responsible for the formation of para-aminohippuric acid from para-aminobenzoic acid [1745]. Estrogens are as effectively conjugated by a mixture of the microsomal fraction with the supernatant as by whole-liver homogenate, while either fraction alone, or other fractions, are not effective [2768].

Several pathways of these transformations exist, with great species variations [386, 3612]. Many substances may be transformed through several pathways simultaneously. Some, such as strychnine, are temporarily stored before detoxification. Blocking of one pathway usually detours reactions into one of the other available pathways. The supply of material used for transformation usually is a limiting factor and determines the pathway used. Other organs may perform the same transformation reaction, as, for instance, hippuric acid formation by the kidney or acetylation by the intestine [312]. This also may represent an alternative pathway. Many reactions have been utilized in hepatic tests.

Degradation. Alkaloids such as quinine [67], ergot alkaloids [1836], and natural and synthetic opiates [248], and probably digitalis [2870] undergo degradation in the liver. Some steroid hormones are also destroyed in the liver, while Dicumarol is partly destroyed [1929].

Oxidation. Examples of oxidation are the transformation of santonin to oxysantonin, cinchophen to oxycinchophen [1996], and cinnamic acid to benzoic acid [3112]. Many of the steroid hormones, such as estradiol [762], progesterone [1249, 2549], and testosterone [3273], are partially inactivated by oxidation.

Conjugation. Almost all the conjugations are combinations with organic or inorganic acids. In the vertebrate phylogenesis, detoxification by sulfate ester formation is the first acquired, followed by glucuronate formation. Only in the more highly developed mammals is coupling with amino acids developed. In liver damage, reversal to a lower type of detoxification may occur, and glucuronate formation may predominate over amino acid coupling.

AMINO ACIDS. Glycine combines with benzoic acid and related substances, such as salicylic acid, para-aminosalicylic acid, and cholic acid, via a peptide binding to form compounds which are readily excreted in the urine. The conjugation of benzoic acid with glycine to form hippuric acid forms the basis of an important hepatic test [2677]. Hippuric acid is mainly formed in the liver in man, but some is also formed in the kidney. In vitro, liver and kidney slices both bind benzoic acid and glycine [350]. ATP [544, 608] and coenzyme A [544] are necessary, with the formation of an intermediate product, benzoyl-coenzyme A. A similar combination of para-aminobenzoic acid has been found [608]. In primates, phenylacetic acid is bound to glutamine before excretion.

Cysteine also participates in conjugation processes. It is usually bound to such substances as halogenated benzenes, naphthalene, and anthra-

cene, via the sulfur atom, and excreted as mercapturic acids. The coupling with amino acids depends upon the amino acid stores in the liver. Liver slices have been shown to be able to carry out oxidation or sulfate or glucuronate formation more easily than the synthesis of mercapturic acid [2301].

GLUCURONIC ACID. Glucuronic acid, derived from glycogen, is conjugated with a series of substances which either contain an alcohol, aldehyde, or organic acid group or are altered in the body to form such groups. This process has been widely used for hepatic tests with many substances, such as camphor, menthol, phenol, or benzene, and their various derivatives [943], guaiacol and paracresol [3571]. Alkaloids, such as morphine, are conjugated with glucuronates only in the liver. The urinary estrogens are often in the form of glucuronates. Benzoic acid and cinnamic acid are bound not only to glycine to form hippuric acid but also to glucuronic acid to form benzoyl and cinnamyl glucuronates, which are excreted as such in the urine [3112]. This pathway of excretion of these substances has long been known, but recently it has been restudied [348] and utilized as a hepatic test [3112]. In liver disease, the normal transformation of benzoic acid to hippuric acid is assumed to be blocked, allowing increased amounts of benzoyl glucuronate to appear in the urine. Similarly, sodium cinnamate in the normal liver is almost entirely oxidized to benzoic acid and excreted as hippuric acid, only a small amount appearing as cinnamyl glucuronate [3112]. In "mild" hepatic-cell damage this glucuronate excretion is increased, because less is oxidized to benzoic acid and excreted as hippuric acid. In severe hepatic-cell damage even the benzoic acid which is formed may be excreted as a glucuronate. Salicylates and halogenated benzenes and phenols [3152] are also excreted as glucuronates. The coupling with glucuronic acid is dependent upon the carbohydrate stores in the liver.

ACETIC ACID. Acetylation concerns primarily the sulfonamides and para-aminobenzoic acid. Also, compounds conjugated with cysteine are then acetylated prior to their excretion as mercapturic acids [1316] (see Amino Acids, above). Coenzyme A is necessary for acetylation [545].

SULFURIC ACID. The halogenated benzenes and phenols are largely excreted as ethereal sulfates [3152], as is indoxyl, derived from indol by oxidation. This is the most primitive and still most extensively used pathway. Sulfate and glucuronate

synthesis may be mutually competitive in detoxification [3238].

Steric Transformation. Steroids may be transformed in the liver by changing the steric configuration. This occurs in the case of cholesterol, which is transformed to cholic acid and excreted in the bile [3088].

Concept of Detoxification. Detoxification is one of the basic functions of the liver. The lack of this function in the hepatectomized dog, as demonstrated by reduced destruction of strychnine or nicotine [2202], results in its rapid deterioration, even if the other known biochemical alterations are corrected. Investigations in recent years, partially with the help of isotopes, have illuminated the basis of hepatic detoxification by conjugation. In principle, two factors are involved: (1) the activity of the enzymes performing the transformation; (2) the availability, or pool, in the liver or in the body of the substance used in the conjugation. Relatively little is known about the responsible enzymes. Their activity is depressed in hepatic injuries, a phenomenon used for hepatic tests. Some of the processes are more sensitive than others. For instance, hippuric acid formation after benzoic acid administration is depressed earlier than glucuronate formation [2677, 3112, 3458].

Glycine has been extensively studied as an example of a conjugating substance for hippuric acid formation. In man, glycine ingestion increases hippuric acid synthesis [2677], and after simultaneous administration of labeled glycine and sodium benzoate, the hippuric acid obtained has a high isotope concentration [3453]. Benzoate ingestion, however, decreases the concentration of free glycine in the plasma [778]. This indicates that the amount of available glycine is one limiting factor in hippuric acid formation. The amount of glycine immediately available for conjugation with small doses of benzoate is 10 mg per 100 gm body weight in the rat [103]. This has been considered the first glycine pool, which is in relatively slow equilibrium with a large pool of glycine and glycine precursors, chiefly outside the liver [103]. Therefore, availability in the liver of the substance for conjugation, rather than availability outside the liver, is of primary importance.

The detoxification of bromobenzene is another example illustrating the pool of the conjugating substance and the fact that depletion of this pool may damage the liver. This substance is conjugated with glucuronate, sulfate, or cysteine [3612].

Parenteral administration of bromobenzene produces hepatic necrosis [1823], which can be prevented by the administration of cysteine or methionine. The hepatic necrosis has been explained by a deficiency of sulfur amino acids in the liver rather than by a toxic action of bromobenzene. In such experiments, only 13 per cent of the bromobenzene given is excreted as mercapturic acid after combination with cysteine. Upon administration of methionine or cysteine, the resulting protective effect is associated with a 23 per cent increase in bromobenzene excreted as mercapturic acid [2635]. This slight increase indicates that protection is the result not of the removal by detoxification via mercapturic acid, but rather of the sparing of the hepatic pool of sulfur amino acids. Moreover, administration of para-dihalogenated benzenes which can not form mercapturic acids does not produce necrosis [1823]. Therefore,

the process of removal, rather than the toxic substance, may damage the liver by depletion of an essential pool.

Whether the damage is the result of increased protein breakdown to replenish the pool or a deficiency of key metabolites remains to be decided. Whatever the cause may be, toxicity and deficiency overlap in such a process, and similar patterns of conditioned deficiency have been suggested for other types of intoxications [949]. In certain situations, therefore, detoxification may take preference over other processes and thus in itself become harmful to the liver.

A practical clinical aspect of the problem of detoxification is the fact that narcotics [488] and barbiturates [2753] are effective in small doses in liver disease and may be harmful in regular doses. This effect of barbiturates has also been demonstrated in animal experiments [2250].

BLOOD AND BILE PIGMENT METABOLISM AND THE ROLE OF THE LIVER

The liver plays a prominent part in the metabolism of the blood pigments, especially in the various pathways of their breakdown. Its function is metabolic and excretory, and it plays a part in the reticuloendothelial system. Both the parenchymal and the mesenchymal cells of the liver participate in these processes, but exactly where many take place is still uncertain. With this reservation, the pigment metabolism is discussed as a unit in this section on the function of the parenchymal cell. The pathways of pigment metabolism, which are closely associated with the pathogenesis of jaundice, are still highly controversial. The disagreement about the nature and the metabolic behavior of the various components is illustrated by the differences in nomenclature. The discussion offered below tries to reconcile the various and sometimes conflicting opinions of the leading modern authorities.

Based on the older studies of van den Bergh [3408], Aschoff [108], McMaster, McNee [2804], Rich [2748], and Fischer [1016], and also strongly supported by the clinical applications of Eppinger [943] and Watson [3502], a concept of bile pigment metabolism was developed which is in harmony with clinical observations and current diagnostic procedures. In recent years, this classical concept, supported and extended chiefly by Watson [3502], has been challenged by Lemberg and Legge [1945], With [3638], and Baumgärtel [185], and, following isotope studies, also by Shemin and Rittenberg [2690, 3639], London [2052], Grinstein and Moore [1287, 1288], and Watson himself [2080]. The modern concept can not yet be integrated, and its clinical applications are still vague. The classic theory will, therefore, be presented first, as the basis of currently applicable hepatic tests. This will be followed by a

more extensive review of the various steps in bile pigment metabolism and the modern concept.

Classical Theory. Hemoglobin, liberated from the red cells chiefly in the spleen, liver, and bone marrow, is taken up by the reticuloendothelial system primarily in the same organs [2748], where it is transformed into a protein-bound bilirubin called hemobilirubin, bilirubin I, bilirubinglobin, or indirect-reacting bilirubin. The last name indicates that this bilirubin gives the diazo reaction of van den Bergh [3408] only after treatment of the serum with alcohol, caffeine, or other substances which supposedly split the protein binding. This indirect-reacting bilirubin does not pass into the urine [3408] but it is taken up by the hepatic cells and secreted into the bile canaliculi, during which process it is transformed into a form called cholebilirubin, bilirubin II, sodium bilirubinate, or direct or prompt-reacting bilirubin [3408], supposedly by separating the protein binding. It is normally excreted with the bile into the small intestine, where it is almost completely reduced by bacterial action to urobilinogen and then oxidized to urobilin. This, with some other bilirubin pigments, is responsible for the color of the feces. Part of the urobilinogen is absorbed into the portal blood system and conveyed to the liver, where most of it is excreted into the bile [2208], or, less likely, reoxidized to bilirubin [3502], thus completing the enterohepatic circulation of the bile pigments. Only small amounts of urobilinogen escape the liver and are excreted in the urine; this is responsible for the weak reaction with Ehrlich's aldehyde reagent normally given by the urine. In obstructive jaundice or hepatitis, the direct-reacting bilirubin passes back into the blood stream, where it may be detected by the van den Bergh reaction without preliminary treat-

ment with alcohol or other substance, since the protein has already been split off. It also differs from the indirect-reacting bilirubin, in that at a certain threshold it passes into the urine. This classic concept is well in keeping with most of the phenomena encountered in the different forms of jaundice [3681] (Fig. 23).

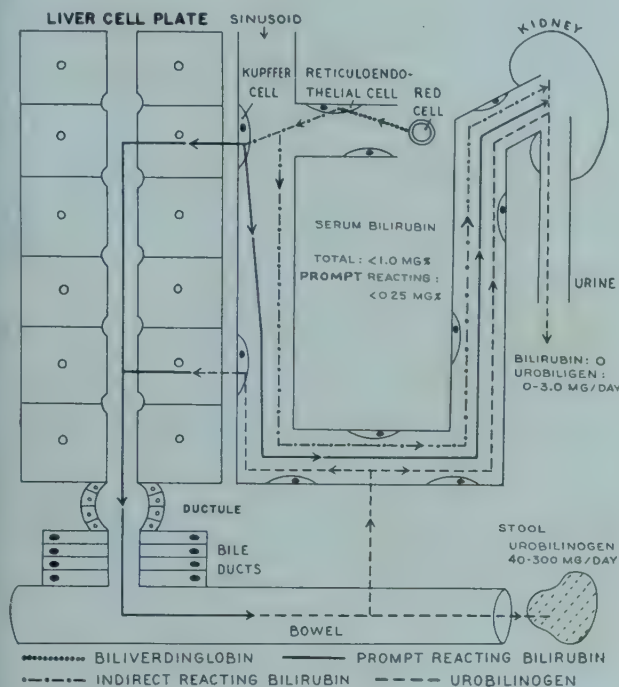


FIG. 23 Normal bile pigment metabolism. (Redrawn from Popper, H., and Schaffner, F.: *Advances of Internal Medicine*, edited by Dock, W., and Snapper, I. 4:357, 1950.)

Nomenclature and Chemistry of Bile Pigments. Appreciation of the recent advances in the knowledge of bile pigment metabolism and their clinical application is made difficult by differences in nomenclature. Lemberg and Legge [1945] have recently introduced a logical nomenclature which, however, has not yet found widespread recognition (Table 5). Therefore, description of the chemical nature of the substance involved appears advisable.

HEME. The basic structure of blood pigments is the pyrrole ring, four of which are combined in either a ring or chain form by methene bridges to constitute a tetrapyrrole pigment [1016]. These tetrapyrrole structures are found as chlorophyll in plants and as pigment constituents in lower animals [1945], while in man and other mammals they occur in blood and bile pigments, as well as in respiratory enzymes. The tetrapyrrolic pigment moiety of hemoglobin is heme, a closed ring with

Table 5 Commonly Accepted and Alternate Names for Various Bile Pigments

Commonly Accepted Names	Alternate Names
Protoporphyrin.....	Hematoporphyrin
Coproporphyrin.....	Enteroporphyrin
Heme.....	Ferroheme, ferroporphyrin
Hematin.....	Ferriheme hydroxide, ferriporphyrin hydroxide
Hemin.....	Ferriheme chloride, ferriporphyrin chloride
Methemoglobin.....	Ferrihemoglobin, hemiglobin
Green hemoglobin.....	Verdoglobin, biliverden globin, choleglobin
Biliverdin.....	Dehydrobilirubin, bilatriene
Mesobiliverdin.....	Glaucobilin, mesobilatriene
Bilirubin.....	Biladiene-a,c
Mesobilirubin.....	Biladiene-a,c
Biliviolin.....	Biladiene-a,b
Mesobiliviolin.....	Biladiene-a,b
Mesobilierythrin.....	Mesobilirhodin, biladiene-a, b
Mesobilirubinogen.....	Urobilinogen A, mesobilane
Stercobilinogen.....	Urobilinogen B, tetrahydromesobilane
Urobilin IXa.....	Lerobilin, stercobilin, mesobilene
Stercobilin.....	Urobilin, tetrahydromesobilene

an atom of bivalent iron at its center, linked to the four central pyrrole nitrogen atoms [1016] (Fig. 24). In heme, a carboxylic acid group is attached to two of the pyrroles and a vinyl group to the two others. Hemoglobin represents a macromolecular pigment protein combination which transports oxygen by virtue of its bivalent iron atom and which consists to a great extent of the protein globin. Variations in the globin account for species differences, whereas the pigment moiety, heme, is the same. The total molecular weight is 68,000, while that of the heme is 616. Globin migrates electrophoretically with albumin and simulates it also in several other physicochemical characteristics, including its molecular size. Originally, one molecule of globin was thought to be attached to one molecule of heme. Now four heme groups are known to be attached to one globin molecule, resulting in about 4 per cent heme in the total hemoglobin molecule [3502].

The iron of hemoglobin is changed in vivo into a trivalent ferric compound by certain intoxica-

tions, with the formation of methemoglobin, which is no longer useful in oxygen transport. By chemical treatment of hemoglobin *in vitro* the heme may

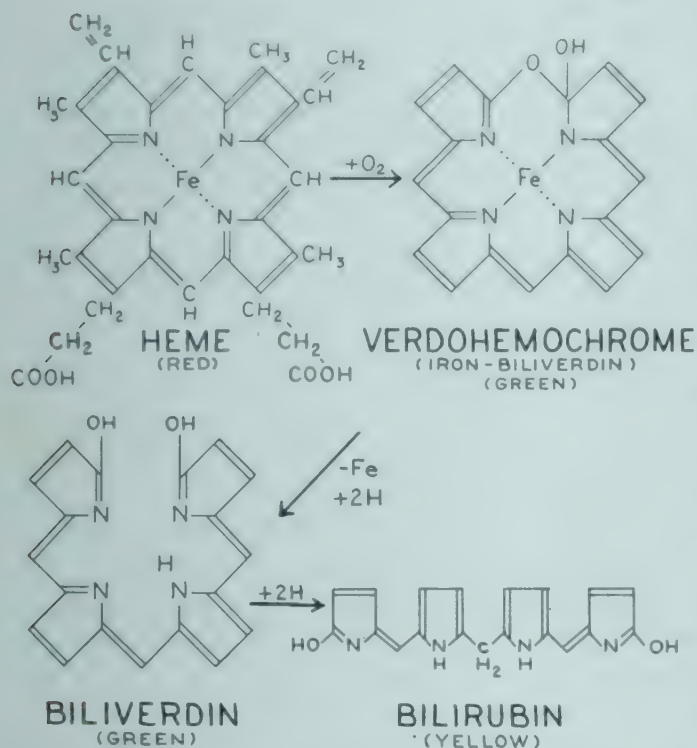


FIG. 24 The formation of bilirubin from heme.

be split off and converted to a ferric compound, the chloride of which is called hemin and the hydroxide, hematin [1945]. Hematin, although not formerly recognized as a physiologic compound, has been found in normal blood, in blood dyscrasias, and in fetal life.

PORPHYRINS. The porphyrins, which are derivatives of the same basic system as heme, are iron-free, tetrapyrrole rings linked by methene bridges (Fig. 25). Hematoporphyrin was originally obtained by splitting off the iron of hematin with concentrated sulfuric acid. It is not a naturally occurring compound. The basic physiologic compound is protoporphyrin IX, which is iron-free heme [3511]. Coproporphyrin has four carboxylic acid groups instead of two and is found in two stereoisomeric forms, I and III [1016]; type I is composed of two asymmetrical dipyrromethenes, as found in heme [1945]. Porphobilinogen is an Ehrlich aldehyde reaction chromogen whose nature is still problematic.

BILE PIGMENTS. The bile pigments result from an opening of the heme ring at the alpha-methene bridge and removal of the iron (and, in many instances, removal of the protein moiety also)

[155, 1945] (Fig. 24). The iron-containing intermediary is a green compound called verdohemochrome [1015]. The iron-free compound is biliverdin, which is green. Its reduced form is bilirubin, which is orange-yellow.

Bilirubin crystallizes in typical rhomboid plates [1945] and is poorly soluble in water, ethyl alcohol, and amyl alcohol. It is soluble in chloroform and in alkaline solutions, although unstable in the latter. It gives the Gmelin reaction, in which a green color is produced on treatment with nitric acid, owing to biliverdin formation, and the diazo reaction (see Chap. 36, Tests Based on Bile Pigment Metabolism). It is usually prepared from ox bile or gallstones [1016]. Since bilirubin is difficult to dissolve in water, the preparation of standard solutions is a problem.

Many reduction products of bilirubin occur biologically. The present nomenclature of these products is somewhat confused. By catalytic reduction of bilirubin with colloidal palladium, Hans Fischer and his coworkers obtained a series of products with variable hydrogenation of the vinyl or methene groups. Included were dehydrobilirubin, mesobilirubin, dehydromesobilirubin, and mesobilirubinogen, which can be oxidized [1016]

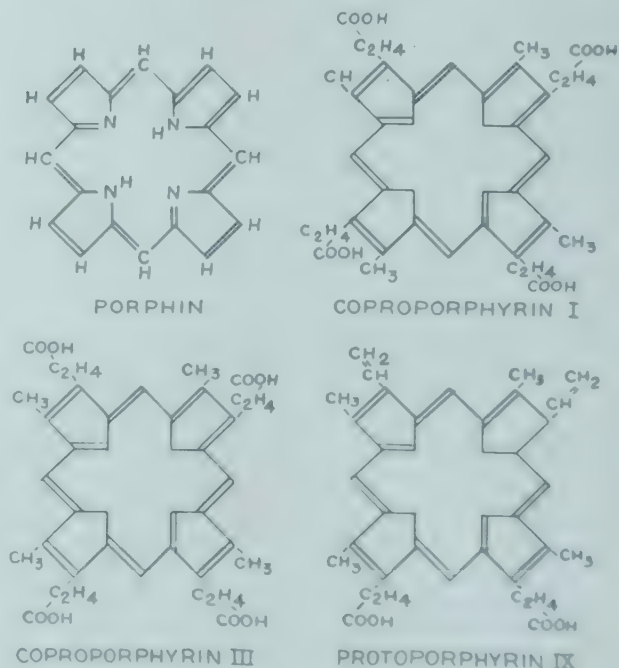


FIG. 25 Structural formulas of porphyrins.

(Fig. 26). Whereas the bilirubin-biliverdin transformation represents a reversible oxidation-reduction reaction, the oxidation of mesobilirubinogen to urobilin IXa and that of mesobilirubin to glaucobilin are irreversible. Watson isolated urobilin

IXa from icteric urine [3502] and stercobilin from feces [3502, 3512], and he prepared urobilin directly from bilirubin [3502].

Stercobilinogen is chemically related to, although not identical with, mesobilirubinogen (Fig. 27). The former has two double bonds and four hydrogen atoms more than the latter [1015] and is, therefore, optically active; it is usually levorotatory [1016], but dextrorotatory forms have recently been isolated [2080, 2965]. The oxidation product of stercobilinogen is stercobilin. Both mesobilirubinogen and stercobilinogen, collectively designated as "urobilinogen," are colorless, i.e., leukoproducts, whereas their oxidation products, urobilin IXa and stercobilin, collectively designated as "urobilin," are brown. Unlike stercobilinogen, mesobilirubinogen and urobilin IXa can be oxidized with ferric chloride and hydrochloric acid to mesobiliviolin and mesobilirhodin [1945]. Using this reaction, a clinical method for differentiating mesobilirubinogen and stercobilinogen in the urine has been developed [185].

Urobilinogen is composed of three chemically, and possibly biologically, different compounds—mesobilirubinogen, stercobilinogen, and *d*-urobilinogen. In feces and urine these compounds behave as a single substance identified by the Ehrlich aldehyde reaction. The qualitative and quantitative determination of urobilinogen has great clinical significance. For the time being, it appears practical in the clinical laboratory to designate

as urobilinogen most leukoproducts which give a red color in Ehrlich's aldehyde reaction [3502], the nature of which is not understood [1016]. The main exception is porphobilinogen, which is insoluble in chloroform, in contrast to urobilinogen.

Urobilinogen is soluble in water and chloroform and is weakly basic. The brownish-yellow oxidized pigments, urobilin IXa, stercobilin, and *d*-urobilin, form fluorescent zinc salts in the Schlesinger reaction.

DIPYRROLES. Dipyrroles, primarily dioxypyrrylmethenes, occur as colorless compounds called "propentdyopents" or as colored ones, "pentdyopents" (Fig. 27). A characteristic color reaction with sodium hydrosulfite has been described for the latter [281]. Similar dipyrrolic compounds, mesobilifuscins, result chemically from splitting mesobilirubinogen [3067, 3068]. They have a tendency to polymerize, they do not crystallize, and their chemistry is poorly understood.

Synthesis of Hemoglobin. Hemoglobin formation consists of two main parts: (1) the formation of the porphyrin tetrapyrrole; (2) the combination of this ring with iron and protein.

Coproporphyrin is the precursor of hemoglobin [1287, 3502] (Fig. 25). Isotope studies have shown that the four pyrrole nitrogen atoms and the eight alpha carbon atoms of the porphyrin nucleus are derived from glycine [2690, 3639]. Coproporphyrin is probably formed in the hematopoietic system, although other sites are possi-

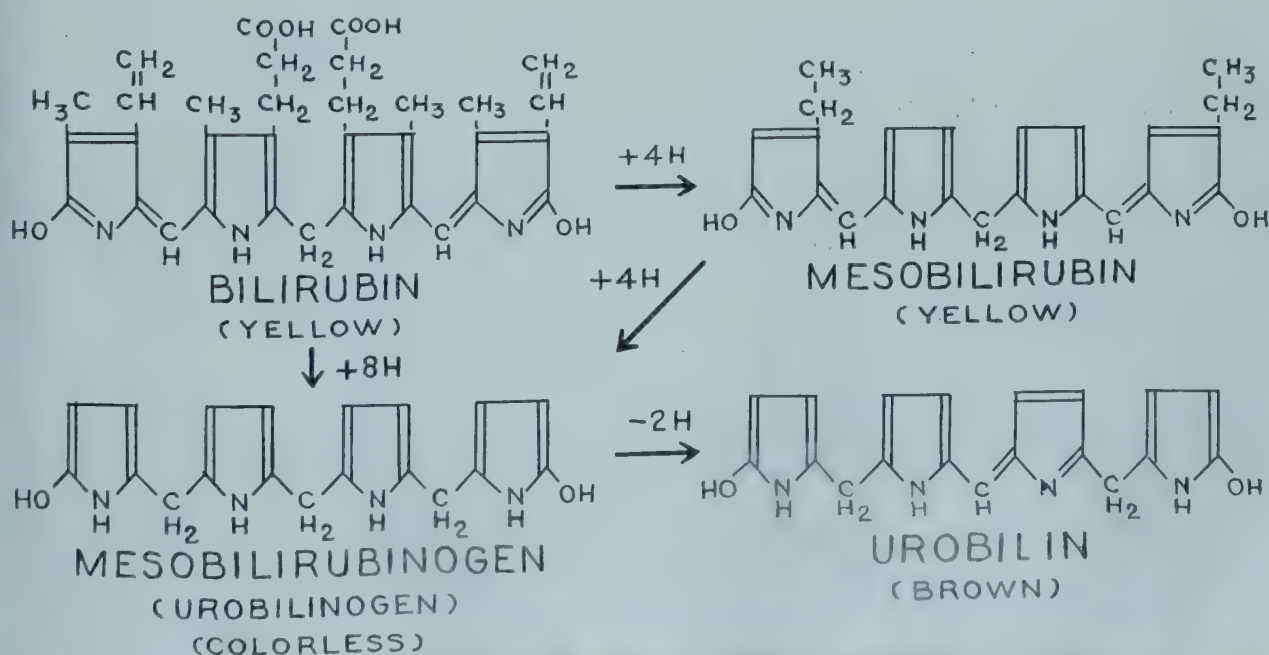


FIG. 26 The formation of urobilin from bilirubin.

ble also. Isomer I is not used for hemoglobin formation and is consequently excreted [804, 1287]. Isomer III is transformed into protoporphyrin and subsequently into hemoglobin [3509]. The evidence for this process is indirect. In various intoxications which interfere with hemoglobin formation, such as lead poisoning and sulfonamide intoxication, the coproporphyrin III excretion frequently increases, and certain microorganisms utilize coproporphyrin III for the formation of enzymes which contain only protoporphyrin [1287]. Bivalent iron combines with protoporphyrin to complete the heme moiety of the hemoglobin, probably in the erythrocyte itself. The globin portion is formed in the liver [3571] and is transported to the sites of hematopoiesis with the plasma proteins [3572]. Globin is bound to the iron of heme by magnetochemical linkages, four heme molecules binding one of globin.

Hemoglobin Breakdown. In the breakdown of hemoglobin, the iron and globin are conserved and reused for the synthesis of hemoglobin, whereas the prosthetic pigment group is discarded (Fig. 28). The pigment breakdown in vivo

follows one of three pathways, the most important of which is the formation of bilirubin.

The second pathway is similar to the breakdown which results from bacterial or chemical action. When free hemoglobin is released as the result of intravascular hemolysis, for instance in hemolytic anemia, blackwater fever, or septicemia, this pathway is utilized [969], though the presence of a damaged liver is said also to be necessary. The bivalent iron is oxidized to trivalent iron, losing its oxygen-carrying ability and changing the color of the compound from red to brown. The resulting hematin was thought to be free of protein but is now recognized to be still attached to protein. This hematin-protein is called "methemalbumin" [969] but is probably hematin-globin. The globin is no longer bound to the iron but is bound directly to the pigment [1945]. Hemosiderin in tissue, in contrast, is pigment-free iron bound to protein. Although hematin-globin is not an intermediary in the normal hemoglobin degradation to bilirubin, it may still be converted into bile pigment [2051], as has been shown by feeding hematin [212, 2051, 2530]. Free hemo-

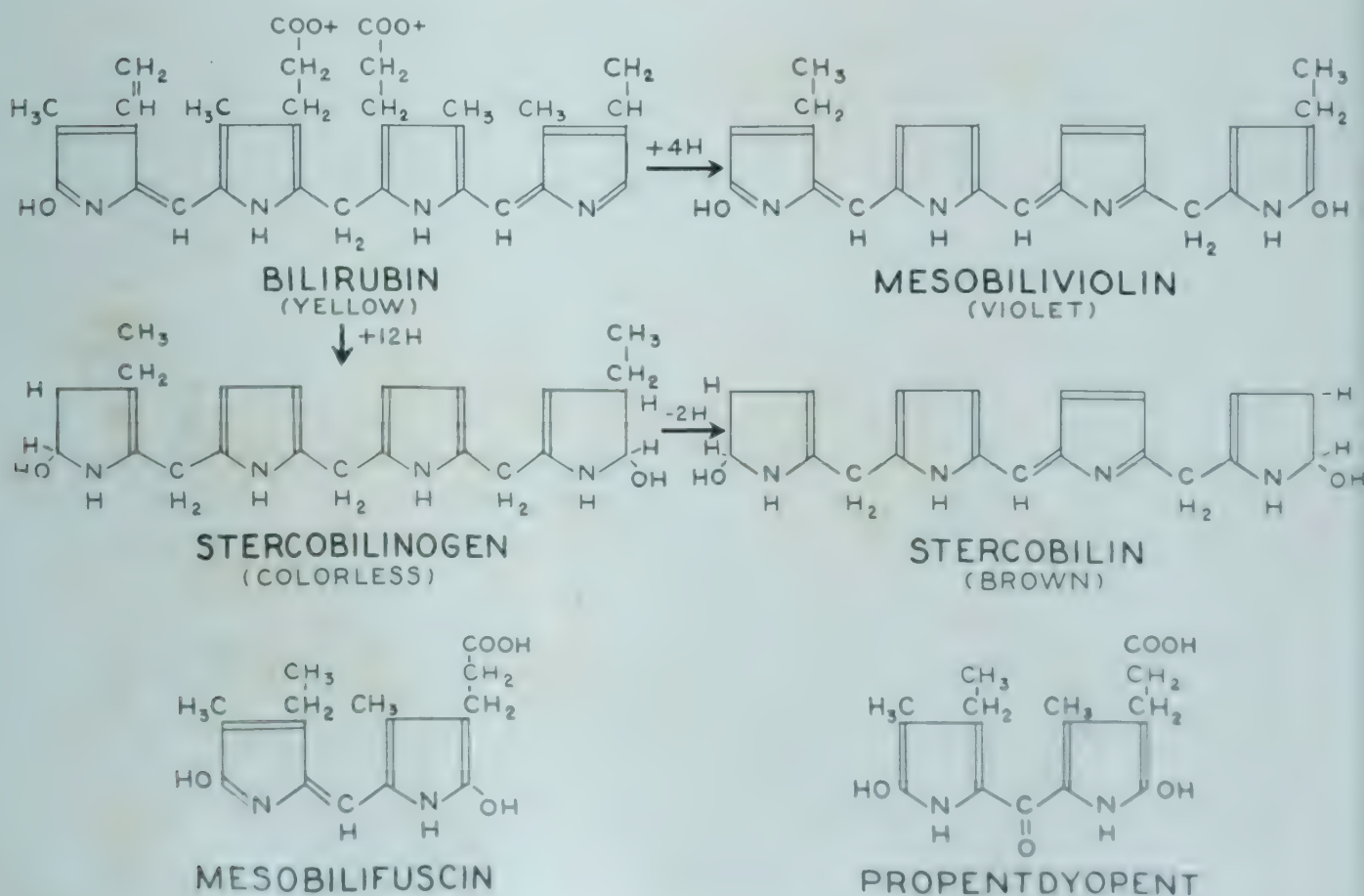


FIG. 27 Formation of stercobilinogen and formulas of dipyrroles.

globin may also lose its iron, as well as its globin, without opening the original pyrrole ring, resulting in the red pigment, protoporphyrin [3502].

A third pathway of the destruction of hemoglobin is a more extensive breakdown of the pigment moiety into dipyrroles, the pentdyopents and bilifuscins (Fig. 27), all of which appear in the stool. These substances have been described in serum and urine in blood dyscrasias and in massive hepatic necrosis [281, 1014]. The absence of jaundice in some forms of extensive hepatic destruction may be explained by an extensive diversion of hemoglobin breakdown into this pathway [3638].

Bilirubin

In discussing the metabolism of bilirubin, the cardinal sign of liver disease, jaundice, which arises when bilirubin is produced or excreted abnormally, must be explained.

Formation. Bilirubin formation, the most important physiologic pathway of hemoglobin breakdown, occurs in the reticuloendothelial cells [2748], primarily in the bone marrow and spleen. Potentially all other organs and probably all mesenchymal cells form bilirubin after phagocytosis of red cells. The hepatic cells also have been claimed to form bilirubin [1953], but this is rather unlikely. The degradation of hemoglobin starts

with an internal rearrangement of the valences of the heme moiety and with oxidation of the alpha-methene bridge but without loss of iron or globin (Fig. 24). This oxidized link is unstable and readily opens. The iron in the green compound formed (verdohemochrome or choleglobin) [1945] is much more readily detached than the iron in hemoglobin [155]. This is not responsible for the "easily split off" iron, which is 4 to 8 per cent of the blood pigment in the circulating red cell, and which is an artefact. It therefore cannot serve as evidence that the transformation of hemoglobin to bilirubin begins in the circulating red cells [1288].

The next step is a change to the iron-free green compound, biliverdin-globin [1945]. A similar compound is found in the bile when bilirubin is oxidized after prolonged biliary obstruction [3502] and may then also appear in the blood. This green oxidized form in the blood causes the green hue of the skin in prolonged biliary obstruction. Intradermal injection of ferrocyanide salt solution is said to make clear the differentiation between biliverdin and bilirubin.

Biliverdin is reduced to bilirubin in many tissues [1945], including the liver [185], and, as such, is discharged into the blood stream. This process occurs only in the carnivores; most herbivorous animals have a green bile which con-

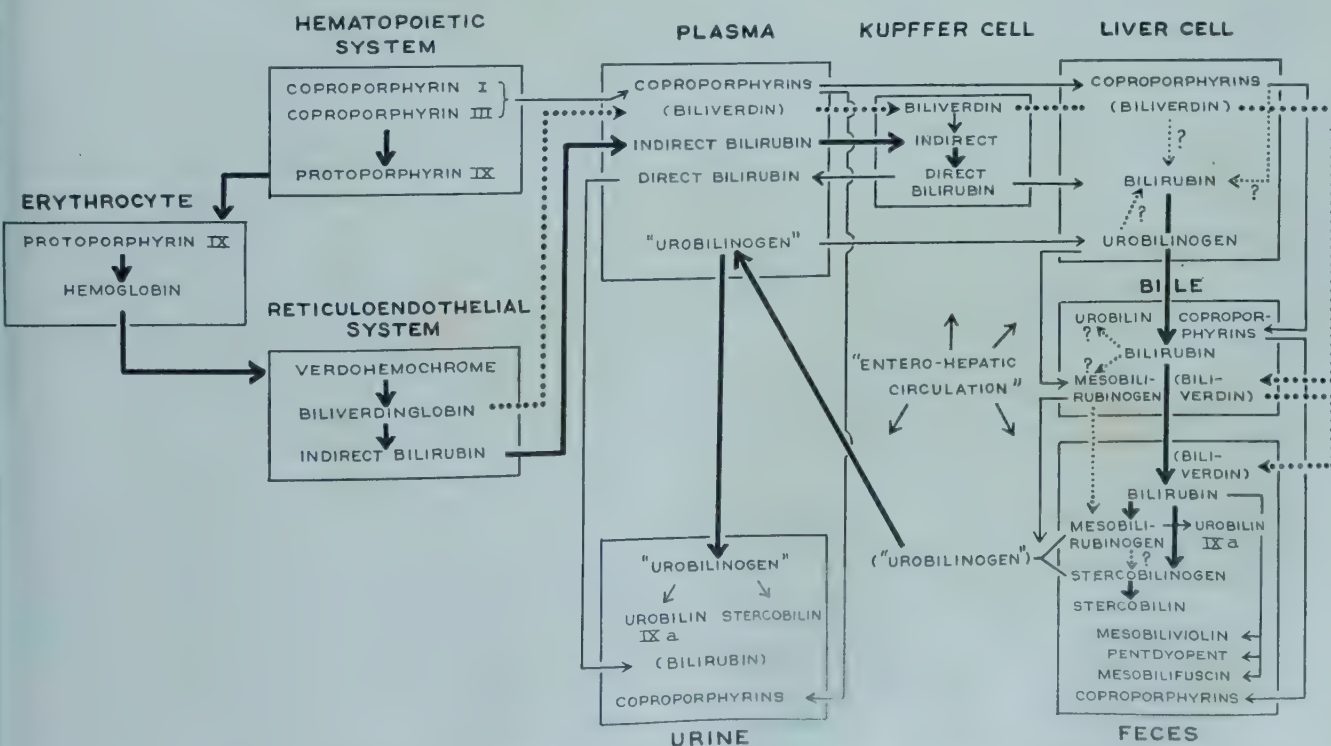


FIG. 28 Pathways of blood and bile pigment metabolism.

tains biliverdin as the chief pigment [185]. An enzyme reducing biliverdin to bilirubin has been demonstrated in the liver and in yellow human bile but not in the bile of herbivores [185].

The protein moiety of hemoglobin is apparently unaltered in the transformation of heme to bilirubin, although conflicting observations have been reported. All bilirubin in the blood stream appears to be protein bound [616, 647], and the protein moiety is said to be albumin rather than globin [1004] or an altered globin [2790]. This problem is further confused by the similar electrophoretic motilities of albumin and globin. Therefore, possibly the newly formed bilirubin is attached to globin, whereas bilirubin which reenters the blood stream from the liver becomes associated with albumin [1004].

Extravascular hemoglobin breakdown follows the same pathway, owing to the action of mobilized reticuloendothelial elements. The resulting tissue pigment, hemotoidin, is identical to bilirubin [1016, 2748].

Biliary Excretion and the van den Bergh Reaction. Bilirubin-globin, released from the reticuloendothelial cells, is taken up from the blood stream by the liver and excreted through the biliary system into the intestine. The liver was formerly thought to be able to excrete excess bilirubin up to twenty times the normal supply [2748]. The narrow limits of such excretion have more recently been emphasized [3638], however, in view of the increase in serum bilirubin observed after removal of two-thirds of the liver [339] and the ease with which jaundice can be produced by bilirubin injection. Bilirubin probably passes from the blood stream to the hepatic cells via the Kupffer cells, although its direct entrance into the bile canaliculi through spaces between the parenchymal cells has been suggested [2544]. The assumption that bilirubin normally passes through both the Kupffer cells and the hepatic cells aids in understanding the pathogenesis of jaundice and the character of the van den Bergh reaction of bilirubin.

Bilirubin treated with diazo reagent (sodium nitrite and sulfanilic acid) gives a red color. This color is readily produced in most icteric serums (direct reaction), but it can be produced in normal serum and in certain icteric serums only if they are first treated with alcohol, caffeine, or other reagents (indirect reaction) [3408]. The direct reaction was thought to be associated with the passage of bilirubin through the liver, during

which it was transformed from the indirect- to the direct-reacting form. The original observations of van den Bergh were subsequently modified, and the sharp differentiation between the direct and indirect reaction became indefinite. In some serums, a color reaction is not obtained immediately after mixing with the diazo reagent but only after several minutes. This delayed direct reaction can be produced without alcohol or caffeine. In other instances an immediate slight reaction becomes much more intense within a few minutes (biphasic reaction).

Considerable controversy developed regarding the chemical, physiologic, and diagnostic significance of the delayed and biphasic reactions [858, 2804]. The development of quantitative methods by use of the spectrophotometer accentuated these difficulties. Transitions between the direct and indirect reaction are now explained by the fact that the acid diazo reagent slowly produces the same effect as the reagents used to transform indirect-reacting into direct-reacting bilirubin in the original van den Bergh reaction [858]. Currently, the prompt diazo reaction developing within 1 minute is attributed to the direct-reacting bilirubin which has passed through the parenchymal cells. All bilirubin reacting after 1 minute is considered indirect [858]. This separation between the 1-minute, or prompt-reacting, fraction and the indirect fraction appears to be the most useful clinically, even though its chemical and physiological basis is not fully established.

Several arguments have been presented for and against two different bilirubins. Prompt-reacting bilirubin was thought to differ from the indirect form (1) by not being extractable with chloroform [1443]; (2) by being readily absorbed by protein precipitates; (3) by being readily changed so that it no longer gives the van den Bergh reaction; (4) by having different absorption spectrums. Moreover, the reaction velocity curve of the diazo reaction indicated the presence of two well-defined components [3502].

The fact that small amounts of prompt-reacting bilirubin are present in normal serum has been taken by some as an argument against the significance of the proposed separation [3010]. Subsequent and more exact studies have failed to substantiate some of the differences described [1278], and a basic chemical distinction had been largely discarded [1786, 1945]. Differences in solubility of the two fractions were said to determine the mode of the reaction. The indirect

van den Bergh reaction has been associated with a persistent globin binding of bilirubin, while prompt-reacting bilirubin has been considered protein-free sodium bilirubinate. Since all bilirubin in serum is protein-bound, either to globin or albumin, the presence or absence of protein can not determine the van den Bergh reaction [621, 647, 1253, 1278, 2226, 3110]. The type of protein binding was thought to be of importance [647, 659]. A serum-albumin fraction containing bilirubin has been isolated which gives the indirect reaction [3638]. The reaction also has been associated with the concentration of bile acids in the serum [172], and bile acids were said to produce a colloidal solution of the bilirubin responsible for the direct reaction [185]. The bile acid theory has its strongest support in the absence of direct bilirubin in hemolytic jaundice, which is associated with low bile acid levels. However, the lack of elevation of the bile acids in hepatocellular jaundice speaks against the dependence of the reaction on the serum-bile acid level. Numerous other theories have been proposed:

1. The physicochemical alterations of the serum itself, and not the passage of bilirubin through the liver, determine the nature of the van den Bergh reaction. Injection of bilirubin elevates only the indirect bilirubin, unless the solution is pretreated with dog plasma and alkali.

2. The speed of the diazotization reaction is determined by interaction of the physicochemical factors [756] and by the amount of bilirubin. The 1-minute point is merely an arbitrary limit, since variable diphasic diazotization curves have been found with equal degrees of jaundice [1786].

3. A catalyst, which is not clearly defined, is formed in obstructive jaundice [1256].

4. Other nonbilirubin derivatives of bile are responsible for the reaction.

Increased prompt-reacting bilirubin in the presence of complete destruction of the liver and the parallel rise of both the prompt-reacting and indirect bilirubin are evidence against the concept of two bilirubins [3638]. The van den Bergh reaction has even been described as a complex chemical phenomenon which should be considered as a curiosity without biological and clinical significance [3638].

In recent years, however, the pendulum seems to be swinging back toward recognition of two types of bilirubin. An indirect-reacting bilirubin has been crystallized. As a globulin complex, it

differs from prompt-reacting bilirubin, which is a metal-containing compound [2406]. Three bilirubin fractions or derivatives have been demonstrated by reverse-phase chromatography [621, 622]. One is most soluble in organic solvents and gives the indirect reaction. The other two are more water soluble and give the direct reaction. The latter two have been numbered simply I and II. Pigment I may be formed outside the liver and is converted to II by the liver. In bile the chief pigment is II, while in the urine it is I. The conversion of indirect-reacting bilirubin to pigment I and of pigment I to pigment II may be impaired in conditions causing interference with bile flow. This may be of diagnostic significance. These pigments differ from bilirubin, mesobilirubin, and dihydromesobilirubin, the only previously known pigments to react with diazo reagent [622].

Most of the arguments against the liver passage theory can be invalidated if one assumes that the conversion of indirect to prompt-reacting bilirubin, whatever its chemical nature, is located in the Kupffer cells [2906, 3502]. This would explain (1) the direct reaction found in massive necrosis (acute yellow atrophy), in which the Kupffer cells are preserved; (2) the presence of prompt-reacting bilirubin in normal serum as a result of "leakage" of bilirubin from the Kupffer cells to the blood, either directly or via the lymphatic vessels; (3) the almost parallel elevation of prompt-reacting and indirect-reacting bilirubin in obstructive jaundice. A block between the Kupffer cells and the parenchymal cells in obstructive jaundice results in the Kupffer cells becoming overloaded with bile pigment and incapable of taking up all the indirect-reacting bilirubin from the blood [2906].

Urinary Excretion of Bilirubin. Except for traces detected only by special methods no bilirubin is found normally in human urine. In some animals it is normally excreted in the urine. The total serum-bilirubin level at which bilirubin is excreted in the urine in appreciable amounts varies [3638]. A renal threshold has been reported to be between 2.0 [3502] and 5.0 [3638] mg per 100 ml serum but occasionally as high as 11 mg per 100 ml. Bilirubin excretion in the urine does not depend only on the serum-bilirubin level, since relatively high blood levels are not associated with bilirubinuria in hemolytic jaundice [756, 3502]. On the other hand, with slight elevations of the total serum bilirubin in other types

of jaundice, significant bilirubinuria may be present [3502]. Even in the same disease, e.g., viral hepatitis, bilirubin may appear in the urine before the serum bilirubin is significantly elevated in the early stages, whereas later high serum-bilirubin levels are not necessarily associated with bilirubinuria.

Since urinary bilirubin gives the direct van den Bergh reaction, at least in fresh urine, the threshold is said to involve only the prompt-reacting portion of bilirubin, the indirect-reacting form being unable to pass through the kidney. This would explain the absence of bilirubinuria in uncomplicated hemolytic jaundice, although other explanations have been offered [1786, 3638]. Discrepancies have been attributed to technical difficulties [3502]. Since the concept of a fixed threshold has to be discarded, important factors are thought to be differences in the ease of urinary excretion between the prompt-reacting and indirect-reacting bilirubin at identical levels [1786, 3708]. The role of bile acids in the urinary excretion of bilirubin is illustrated by the failure of intravenously injected bilirubin to appear in the urine unless bile acids are simultaneously injected. Supposedly, bile acids transform bilirubin from the indirect to the direct-reacting form prior to excretion [185]. A specific effect of the kidney has also been postulated, supported by the absence of bilirubin excretion in renal failure in conditions otherwise associated with bilirubinuria. Since the ability of the kidneys to excrete bilirubin is very limited [3638], variations in urinary excretion are probably explained better by variations in renal function than by changes in the physicochemical state of the bilirubin. The fact that bilirubin is more easily excreted in obstructive jaundice than in parenchymal jaundice also has been explained by different renal clearances [2775].

Bilirubin Degradation. **HEPATIC.** The chief pathway of excretion of bilirubin is into the bile. Reduction of bilirubin to mesobilirubinogen may occur to a small degree under normal circumstances in the liver or bile [185], but the hepatic transformation of bilirubin has been questioned [2896]. In gallbladder bile, transformation may occur in man owing to stagnation of the bile, explaining the presence of a substance giving a urobilinogen reaction in bile [1945, 3638]. Urobilinogen in infected bile is apparently produced by bacteria [943] and is dextrorotatory, in contrast to the usual form [2080, 2965].

ENTERIC. Bilirubin in the intestinal tract is reduced by bacterial action to mesobilirubinogen via dihydrobilirubin and mesobilirubin [3502] (Figs. 26, 28). Following administration of antibiotics that temporarily sterilize the intestinal tract, dextrorotatory urobilinogen is present and may also be an intermediate in the reduction of bilirubin to mesobilirubinogen [2080]. This *d*-urobilinogen, originally found in bacterial infections of the biliary tree, is the predominant fecal pigment after discontinuation of antibiotic therapy. The further reduction of some mesobilirubinogen directly to stercobilinogen has been demonstrated by isotopic studies [2079, 2080, 3502]. The remaining mesobilirubinogen is oxidized either in the colon or outside the body to urobilin IXa [2080]. Similarly, stercobilinogen is oxidized to stercobilin, and *d*-urobilinogen to *d*-urobilin.

A dualistic theory of reduction of bilirubin has been presented [185] in which it is suggested that mesobilirubinogen is formed enzymatically in the liver, and that stercobilinogen is formed by bacterial action in the intestine. This has been challenged, since mesobilirubinogen is a precursor of stercobilinogen (Fig. 28). Both are equally absorbed from the large intestine and returned to the liver in the enterohepatic circulation, with only a trace escaping in the urine. These two derivatives apparently have equal physiologic and diagnostic significance. The concept that bilirubin is mainly reduced in the intestine by bacteria is supported by the disappearance of the urobilinoid pigments from the urine after antimicrobial therapy [2896] and after ligation of the common bile duct in dogs. Some observations are not in keeping with this concept [185, 1945]:

1. In some instances of jaundice, more urobilinogen appears in the urine than can be formed from the bilirubin excreted into the intestine.
2. In hemolysis, urobilinoid pigments may appear in the urine earlier than would be expected if they are products only of intestinal transformation.

Not all bilirubin is transformed into the urobilinoids; part is broken down to the dipyrroles—pentdyopents, bilifuscin, and mesobilifuscin. These substances may exceed the tetrapyrroles, stercobilin and urobilin IXa, in amount [3067, 3068] and are also partly responsible, in addition to urobilin, stercobilin, and other less well-established pigments, for the brown color of the feces.

Urinary Excretion of Urobilinogen. Only traces of urobilinogen are found in the peripheral blood,

and not more than 0.5 mg per 100 ml has been observed even in the presence of severe jaundice. Urobilinogen can be demonstrated in fresh urine in small amounts normally [3502], and in increased amounts in concentrated urine, as well as in various disorders. The demonstration of urobilinogen in the urine has become a widely used clinical procedure, based on the extensive work of Eppinger [943] and Watson [2964, 3502, 3515]. Urobilinogen appears in the urine together with its oxidized colored form, urobilin. Some urobilin is formed secondarily by oxidation outside the body by light or bacteria, or in the bladder, by bacteria. A separation of the two main components of the urobilinogen group, mesobilirubinogen and stercobilinogen, has been attempted with the ferric chloride reaction [1945], which is given only by mesobilirubinogen [185, 1945, 3502]. The diagnostic value of this separation has not been demonstrated.

Porphyryns. A number of substances of different physiologic significance are classified as porphyryns (Fig. 25). They include (1) *in vitro* artefacts, such as hematoporphyrin [1945]; (2) hemoglobin precursors such as coproporphyrin III and protoporphyrin IX [1287] and by-products, such as coproporphyrin I; (3) intestinal hemoglobin-breakdown products, such as deuteroporphyrin, mesoporphyrin, and probably a coproporphyrin; (4) the uroporphyrins found, in addition to increased amounts of coproporphyrins, in the urine in idiopathic porphyria [3511].

Coproporphyrin I, as a by-product of hemoglobin formation, is discarded via the bile and urine and is the predominant porphyrin in normal urine [3509]. In types of jaundice with reduced biliary excretion, it is diverted from the bile to the urine, increasing the urinary concentration of coproporphyrin I [3508]. Isomer III as a precursor of hemoglobin and respiratory enzymes seems to be formed in excess of the requirements. It is also excreted in the urine and bile, the amount depending on such factors as weather and water supply [3509]. Urinary coproporphyrin III excretion is increased in (1) disturbances of hemoglobin formation such as aregeneratory anemias; (2) toxic conditions such as lead poisoning; (3) alcoholism and liver disorders associated with it [804, 3511]. The ratio between the urinary concentration of the two isomers may have diagnostic significance as to the type of liver disease.

Porphyria. Porphyrias are clinical conditions characterized by alterations of porphyrin metab-

olism and by excretion in the urine of uroporphyrin, recognized by its fluorescence, and porphobilinogen, which reacts with Ehrlich's aldehyde reagent [3503]. The porphyrias can be divided into a form in which the disturbance has been localized in the bone marrow and one in which it is in the liver. The erythropoietic form, which is also called congenital or photosensitive porphyria, is characterized by photosensitivity, which appears shortly after birth, and the presence of coproporphyrin I and the absence of porphobilinogen in the urine. In hepatic porphyria, the amounts of porphyrin and porphyrin precursors in the liver are greatly increased, especially in hepatic-cell nuclei [2932]. Laboratory tests usually indicate some impairment of hepatocellular function. Jaundice and spider nevi have been observed, and cirrhosis was found in some cases [3503], although the relation of porphyria to hepatic injury is not established. Hepatic injury may be the result rather than the cause of the disturbance.

TYPES OF HEPATIC PORPHYRIA. Several forms of hepatic porphyria have been recognized [3503]. The one which is most common, the acute intermittent form, is apparently inherited with mendelian predominance. Attacks of variable duration occur, with abdominal and neuropsychiatric manifestations. The abdominal symptoms are pain, often colicky in type, and constipation. Sometimes the symptoms call for the differential diagnosis of a surgical condition. The neurologic manifestations include peripheral neuropathy, pain in the extremities, paralysis, and sometimes bulbar manifestations. The psychiatric findings vary from hyperirritability to frank psychoses. The urine contains both porphobilinogen and a mixture of uroporphyrins I and III, which sometimes has been called the "Waldenström porphyrin."

In the cutanea tarda type, photosensitivity appears in the fifth or sixth decade, with eczematoid dermatitis, the formation of bullae, and hypertrichosis. The urine contains uroporphyrin of the Waldenström type but no porphobilinogen.

A mixed form occurs which exhibits both cutaneous and abdominal or neurologic manifestations. In this form hepatic involvement is most conspicuous.

Porphobilinogen is sometimes excreted in the urine in various liver diseases without manifestations of porphyria. It is separated from urobilinogen by the chloroform solubility of the color that develops with Ehrlich's aldehyde reagent.

The excretory function concerns the formation of bile and the excretion of exogenous substances, particularly various dyes. For the hepatic cells, bile formation includes the transport of bilirubin in aqueous solution from the blood to the bile canaliculi, probably with the help of enzymes that become depressed under abnormal circumstances [1374].

BILE FORMATION

Bile is partly a secretory product of importance in intestinal digestion and partly a waste product for discharge of useless material into the stool. As our knowledge increases, we include fewer substances in the latter group. Bile consists of various substances in aqueous dispersion, some of which, such as cholesterol or bile pigments, are not readily soluble in water and are kept in suspension only by the presence of stabilizing and dispersing agents such as bile acids and fatty acids. The physical and chemical characteristics of hepatic bile mirror hepatocellular function better than the chemical constitution of the blood. Hepatic bile undergoes significant alterations in the biliary passages. Most of the available descriptions of bile refer to gallbladder bile. In this section emphasis is given to the formation and constitution of the bile as it originates from the hepatic cells.

Physical Characteristics of Liver Bile. The total volume of bile secreted by the liver is not well established, either in man or in animals. It is dependent upon sex, surface area, body weight, and the amount lost through bile fistulas, from which most of the available information is obtained [642]. Under these circumstances, bile constituents, especially bile acids, which are

normally absorbed from the intestinal tract, are lost and the physiologic stimulation for bile production is altered. In addition, variation of food intake influences the secretion. In bile fistula dogs, the volume of bile is doubled by feeding and redoubled by return of bile to the animal [1827]. The average output in man is 300 to 700 ml in 24 hours [1543]. In a healthy person with a normal liver, an excretion between 10.5 and 11.0 ml per kilogram of body weight per 24 hours was recorded, with a fairly continuous flow during the day and a decreased flow when the patient was sleeping [3731]. The excretion of 13 to 29 ml per kg per day in the dog and of comparable amounts in other animals has been reported [1543]. All these figures are probably too low. With intact enterohepatic circulation of the bile acids, the excretion of liver bile is between 700 and 1,200 ml per day in the normal adult on an average diet.

Fistula bile in man is usually light orange-yellow, but its color depends on the pigment concentration [1543]. Bilirubin causes an orange color, and biliverdin a green color. The color varies in different animals. In the guinea pig it is colorless, while in the ox it is green. On standing, the color changes from orange to green and brown, owing to oxidation.

The specific gravity of human liver bile lies between 1.008 and 1.015. The freezing point of bile is between -0.56 and -0.61°C , like that of blood [1543]. The pH of the bile varies considerably; in general it is somewhat alkaline. In man, it is between 5.70 and 8.60. Variations are largely caused by variations in the diet. Meat intake shifts it to the acid side, and vegetable intake to the alkaline side. Many other physical properties have been recorded, without uniform-

ity [1543]. Gallbladder bile is slightly acid, with a pH of 5.0 to 6.0.

Chemical Constitution. The reports on the chemical constitution of liver bile are quite divergent, not only in different animals but also in the liver bile of man. Most of the findings were recorded many years ago [1543]. The average water content in the liver bile is 97 to 98 per cent. Of the 2 to 3 per cent solids, almost half are bile salts [1996]. The fatty acid concentration was shown to be about 100 to 130 mg per 100 ml in a patient with a bile fistula and a normal liver [1611]. The total cholesterol concentration is between 50 and 160 mg per 100 ml [1543, 1611], although in gallbladder bile it is 100 to 400 mg per 100 ml and may rise to 900 mg per 100 ml. Much lower amounts (8 to 18 mg per 100 ml) are found in rat bile [1101] and dog bile, while in hog bile the concentration is high and reaches the serum concentrations. Phospholipids in human bile vary from 1 to 2 mg phosphorus per 100 ml, while somewhat more is found in dog bile [2904]. Bile acids and fatty acids keep the cholesterol in solution [812]. Bile has approximately the same composition of minerals as blood. Sodium and chloride are the ions found in highest concentration (Fig. 19). Bicarbonate is twice that of plasma in gallbladder bile, but not in liver bile. In addition, calcium, magnesium, iron, copper, phosphates, carbonates, and traces of zinc and cobalt are found. [1543]. The concentration of calcium in liver bile is slightly below that of serum. Data on the concentration of minerals are misleading in view of variable filtration and reabsorption by the liver.

BILE PROTEINS. Significant amounts of protein, mainly albumin, are found only under pathologic circumstances (albuminochole [943]). Mucoproteins as well as mucin are present in the bile as secretory products of the adnexal glands of the bile passages and gallbladder. Urea, uric acid, creatinine, amino acids, nonprotein nitrogen, and sugar appear in the bile in concentrations comparable to those in the blood [1836]. The biliary sugar concentration is increased in diabetes and after epinephrine administration, and decreased after insulin administration [1543].

ENZYMES AND VITAMINS IN BILE. Phosphatase has been demonstrated in the bile [482], but its activity does not parallel that of serum phosphatase. Amylase, found in gallbladder bile, may be the result of pancreatic reflux (see Reflux between Pancreatic and Biliary Ductal System,

under Interrelation between Gallbladder and Sphincter of Oddi, Chap. 16). The fat-soluble vitamins A and D, as well as water-soluble vitamin C and some members of the B complex, and exogenous and endogenous estrogens and androgens [480] are found in bile.

BILE ACIDS IN BILE. No reliable figures are available with regard to the total amount and concentration of bile acids excreted in the bile. The concentration varies from 0.22 to 1.83 gm per 100 ml in human biliary fistula bile [3125]. In the dog this varies from 0.02 to 1.13 gm per kg per day [74]. Bile acids are excreted chiefly as sodium salts of cholic acid. They are mainly conjugated either as taurocholic acid or glycocholic acid [817]. Under normal circumstances, the majority is glycocholic acid, although variable ratios between the two are reported [1996, 3125]. Under abnormal circumstances, the taurocholic acid may be excessive [3125]. Large amounts of free bile acids are found in hepatic diseases [74].

BILE PIGMENTS IN BILE. The concentration of bile pigment, mainly bilirubin, varies. The total amount in man is estimated to be between 0.5 and 2.1 gm per day [1543]. In biliary fistula bile in man, it is 5 to 7 mg per kilogram of body weight [3698], with a concentration of 2.3 to 70.0 mg per 100 ml [1996, 2825, 3125]. In the rat, 1.20 mg per 100 ml has been reported [1101].

EXOGENOUS SUBSTANCES IN BILE. Many exogenous substances are excreted in the bile. Organic poisons such as strychnine or quinine are excreted in combination with bile acids, possibly as part of a detoxifying process. Many drugs are excreted in the bile, such as aureomycin [3693], penicillin and streptomycin [3692], caffeine, salicylates, sulfonamides, curare [1543], digitoxin [2870], and mercurial diuretics [2723].

Bacteriology of Bile. Early observations on acholic stools and on fermentation and putrefaction led to the assumption that bile is antiseptic [1543]. In more recent bacteriologic studies bacteria were cultured from the gallbladder wall in 60 to 75 per cent of the specimens examined [70], although in acute or chronic cholecystitis cultures are usually negative. Improved techniques demonstrate the in vitro growth of such bacteria as typhoid and cholera organisms in media containing bile. The main exceptions are the pneumococci, against which bile has specific cytologic power. Many common strains of bacteria have been identified in human bile [1543]. Those most frequently found are *Escherichia*

coli, *Bacillus proteus*, *Aerobacter aerogenes*, *Bacillus pyocyaneus*, and *Bacillus Welchii* [2888]. Infection or stones in the biliary tract increases the incidence of bacteria in the bile. In one series, *E. coli* was cultured from the bile in every case with cholelithiasis [2888]. An organism of specific interest is the typhoid bacillus, which is harbored in the bile in the carrier state. Cholecystectomy effectively removes this source of infection [2032].

Aerobic and anaerobic bacteria, which have reached the liver primarily from the intestinal tract through the portal vein or from the general circulation via the hepatic artery, are excreted through the bile. Some types of bacteria are excreted only after injury to the hepatic cells. Intravenously injected *E. coli* or streptococci appear in bile in large numbers only after damage of the hepatic cells. Bacteria have been found during general anesthesia but not during local anesthesia [2625]. Excretion of bacteria in the bile is not the result of functional activity of the liver but rather an expression of a breakdown of the barrier between the blood stream and the bile. This is particularly evident since the virulence of the bacteria is not influenced by this excretion [2625]. Whether the intact gallbladder mucosa discharges bacteria into the bile, unless the cystic artery or cystic duct is occluded, is questionable. The biliary tract may contain hematogenously disseminated bacteria in bacterial endocarditis.

Factors Influencing Bile Formation. Cholagogues increase the rate of secretion of bile from the hepatic cells into the bile canaliculi. This action has to be differentiated from the action of cholagogues, which expel bile from the biliary passages into the duodenum. Two types of cholerisis are described. In one, the volume of the bile is only slightly increased, but the bile is definitely thicker, i.e., it has increased viscosity. It contains more nonvolatile solids and is rich in bile acids. The other, hydrocholerisis, implies increased biliary volume with a relative decrease of the concentration of bile acids and solids, although the total output is also increased. Under such circumstances bile is thin but the specific gravity is little altered. The choleretic effect of any substance is ascertained by means of complete biliary fistulas. Since these are rarely produced in man and then hardly under normal circumstances, reliable data are scanty. Although the total amount of biliary substances is con-

siderable, biliary secretion does not influence the body fluids as radically as urinary secretion. Bile formation is influenced by the same factors as urine formation, but it does not respond so readily to changing needs of the organism.

FILTRATION VS. SECRETION. Comparison of the plasma concentration and biliary excretion by clearances similar to renal clearances has recently been made for bile formation [643]. Substances such as electrolytes, urea, creatinine, and cholesterol, which have a relatively high blood level, are excreted in the bile in low concentrations. The bile/plasma ratios of these substances are nearly constant, and the clearances are low. These substances are apparently excreted by a filtration mechanism, without specific vital intracellular forces. During hydrocholerisis, as induced by some bile acids, the clearance of these substances rises. In contrast, some substances, including bilirubin, para-aminohippurate, penicillin, and Bromsulphalein, have relatively low plasma and high biliary levels. The bile/plasma ratio varies from substance to substance, and active secretion is assumed. Hydrocholerisis depresses the ratio [643].

INFLUENCE OF CIRCULATION. Although bile flow is a function of blood flow [381], circulation has a complex effect on the bile flow [1996]. Increased portal blood flow increases the bile flow, whereas sudden occlusion of the portal vein decreases the formation of bile but does not stop it. Eck-fistula dogs secrete only half the normal volume of bile. An increase in the hepatic arterial pressure decreases the bile flow, whereas occlusion of the hepatic artery does not necessarily prevent its formation [467] and may actually increase it. In partial support of earlier observations, direct measurements with a thermostromuhr demonstrated that hydrocholeretics such as sodium dehydrocholate and cinchophen given intravenously increase the hepatic arterial blood flow [1291]. Choleretic substances, which only moderately increase bile volume but greatly increase the total solids, do not influence the total bile flow. Anoxia decreases the flow of bile [2943] but does not significantly alter bile salt excretion [2146]. Diathermy may increase the flow of bile by increasing the blood flow.

INFLUENCE OF BILE ACIDS. The chemical nature of bile acids influences bile formation. The naturally occurring nonoxidized, conjugated bile acids produce a true cholerisis. Hydrocholerisis is produced by the oxidized bile acids, such as

dehydrocholic and ketocholanic acids, the non-conjugated forms being more efficient than the conjugated ones [239]. When a choleretic effect is desired for therapeutic purposes, the less toxic, oxidized, nonconjugated, and often synthetic form is used. In hepatic congestion, hydrocholeresis cannot be produced [3295]. In bile excreted under the influence of choleretics as well as of hydrocholeretics, the total amount of bile acids is increased. This is, however, the result of increased uptake from the blood and not of increased formation of bile acids, which may even be suppressed. Cholesterol concentration of the bile is somewhat increased, probably owing to its better solubility in the presence of bile acids. The total bile pigment excretion is normally not influenced by choleresis; the concentration is even decreased during hydrocholeresis [238]. In hyperbilirubinemia, the total amount may be increased.

NERVOUS REGULATION. Nervous factors which influence bile flow are related to blood flow. Section of the splanchnic or hepatic nerves increases bile flow for several hours, while stimulation of these nerves decreases it. After section of the splanchnic nerves, occlusion of the hepatic artery increases the bile flow even further [3295]. Distention of the colon by electric stimulation of the colonic, mesenteric, and pelvic nerves decreases the bile flow [1206]. This reflex is elicited after decentralization of the celiac ganglion and of the vagi and splanchnic nerves and excision of the lumbar sympathetic chain [3487]. Therefore, the celiac ganglion was assumed to be a true or pseudo reflex center for temporary inhibition of hepatic bile flow with thoracolumbar sympathetic or prevertebral autonomic pathways. The vagus nerves have both excitatory and inhibitory effects [3295]. Emotional factors were ascribed a role in the secretion of bile even in antiquity. This has given rise to such terms as "melancholy" (black bile) and "biliousness." More recently it was shown that stress or fear increases bile flow, while rage suppresses it. Rest or sleep results in a diminution of bile flow to two-thirds that during activity [1842]. Hunger increases the flow of bile, while nausea or vomiting decreases it.

HORMONAL INFLUENCE. Secretin, an extract of the upper intestinal mucosa which stimulates intestinal and pancreatic secretion, influences bile formation in the dog and cat even in the absence of the entire gastrointestinal tract, pancreas, and spleen [3295]. In man, it has a hydrocholeretic effect [1301].

INFLUENCE OF DIET. The availability of a patient with a total external biliary fistula and a normal liver following cholecystectomy offered the opportunity to study choleresis as a result of diets [1612]. The greatest increase in bile flow was produced by a high-protein diet, confirming many previous reports based on animal experiments [1827]. High-fat diets or mixed hospital diets had half the effect, and pure carbohydrate diets were without effect. Although carbohydrates did not quantitatively alter the bile excretion [1611], intravenous injection of hypertonic glucose solution suppressed the bile flow, supposedly because of glycogen formation, since nonmetabolized sugars had no effect [1613]. Olive oil was without effect, but oleic acid acted as a choleretic and even increased the choleretic action of bile itself [3730]. Dietary cholesterol had no effect on bile formation. These findings in a single person agree fairly well with observations in animals that protein is the most powerful choleretic, and fat a weaker one, in contrast to its powerful cholagogic action, while carbohydrate has no choleretic effect.

EFFECT OF DRUGS. Many substances have choleretic activity [524, 1306, 2179]. Cinchophen was described as a choleretic drug many years ago. Its effect varies, however, in different species [2179], and in some, cinchophen even leads to a depression of synthesis and secretion of cholic acid [82]. Cinchophen produces hydrocholeresis with increased excretion of cinchophen itself in the bile [81]. Salicylates [2933], naphthol [2207], camphor and aromatic oils, and podophyllin [943] are supposedly choleretics. Choleretic action has been attributed to many substances, especially in the earlier literature, but many of them, such as calomel, have not been found, on careful pharmacological examination, to have such action [2933]. Morphine is not choleretic. Histamine and insulin lead to increased bile production [2481].

DIURNAL VARIATIONS. More bile is excreted during the day than during the night. The diurnal variations of the bile flow are the result of the interaction of many of the factors described. The most important is the intake of food, especially protein, during the day. Liver, particularly because of its bile acid content, and lean meats are the most powerful stimulants, which, when enhanced by secretin, lead to choleresis with equal participation of water, cholates, cholesterol, and pigments [1301]. If a continuous flow of thin bile is indicated for therapeutic reasons, frequent high-protein feedings are useful.

Abnormal Bile. Relatively little is known about the constitution of hepatic bile under abnormal circumstances, especially in the presence of an acutely or chronically damaged liver. Under certain circumstances, secretion of large amounts of a highly pigmented bile with low bile acid concentration (cholorrhagia) has been reported. On the other hand, bile secretion is reduced in carbon tetrachloride intoxication. In liver damage, biliary cholesterol and bile acids are reduced. Rats with choline-deficient fatty livers produce a smaller amount of bile in which the lipid level is reduced [631], contrary to earlier reports. More is known about the constitution of bile after the release of biliary obstruction. The excretion of bile acids in human bile is delayed for 2 to 10 days, and in experimental animals for 5 days [238, 2721]. As a rule, in liver damage bile pigment secretion is low. However, whether a pigment-free white bile has ever been found is questionable, despite occasional reports of such a phenomenon [943].

Histologic Changes during Bile Secretion. The histologic demonstration of bile secretion is still an unsolved problem. The Golgi apparatus has been considered the site of the formation of bile pigment; it is periodically destroyed and re-formed during bile formation. The mitochondria may also be related to this process. Barium chloride fixation of the bile pigment, with subsequent staining by a modification of Mallory's aniline blue method, reveals a zonal distribution of intracellular bile. Diurnal rhythm is noted and is apparently inversely related to the glycogen content of the liver, because during the secretory phase, glycogen-poor hepatic cells contain many secretory granules of bile [1054]. At the height of the secretory phase the bile canaliculi, especially those on the periphery of the lobules, are dilated and thick-walled, and contain bile. When secretion is at its lowest point, they are thin-walled and collapsed. Hydrocholeretics produce a picture similar to that seen at peak secretion [586].

Function of the Bile. Bile is necessary for life. It acts as a medium for excretion, especially of bile pigments; as an aid in the absorption of digested food; as an activator of intestinal and pancreatic lipolytic and proteolytic enzymes; and as a regulator of the bile flow. The bile acids fulfill most of these functions.

Bile influences the constitution as well as movement of the intestinal contents, the bile acids again being of major importance. In their absence, fecal fat is increased, in addition to fatty acid soaps,

which form in the alkaline medium produced by pancreatic and intestinal juices [1295]. This excess fat is not necessarily dietary fat but may result from fat secreted by the intestinal tract and not reabsorbed. Bile aids in the regulation of the salt and water balance of the body, and the biliary excretion of alkaline substances influences the acid-base balance [363]. The blood carbon dioxide and chloride levels, however, are not necessarily altered in dogs with chronic biliary fistulas [74]. Dehydration occurs in all animals with external bile fistulas. Death occurs in months in dogs [3571], guinea pigs [3125], and rats [631] with external bile fistulas, primarily because of water and salt deprivation and the rapid development of peptic ulcers. These observations should be considered in postoperative management. Prolonged absence of bile leads to anorexia and weight loss, with intestinal disturbances, anemia, hemorrhagic tendencies, and osteoporosis [3571].

The Therapeutic Value of Bile. Dogs with external fistulas may live as long as 4 years if bile is given by mouth. Whole bile has been used therapeutically for many years, but now its action is known to be the result of bile acids. Two main types of effects may be obtained: (1) an effect upon the intestine, the more important aspect of which is the improved absorption of fat and fat-soluble vitamins, the less important being laxative action; (2) an increase of bile flow in liver, biliary tract, or gallbladder. If the first effect is desired, oral administration of bile acids is the therapy of choice. If the effect upon the liver and the biliary passages is desired, intravenous, as well as oral, administration is recommended. The intravenous route is indicated where a rapid flush is desired; otherwise oral administration suffices in view of the ease of absorption of bile acids. For intravenous use the less toxic, oxidized bile salts are preferred to the naturally occurring nonoxidized forms, which are hemolytic. In oral administration, systemic toxicity from the nonoxidized salts is less likely and the only important side effect is intestinal irritation. After relief of biliary obstruction, acceleration of bile formation, for which administration of bile acids has been suggested, may be desirable. Whether this increases the excretion of pigment or enhances recovery is not established. In infections of the intrahepatic and extrahepatic bile ducts (purulent cholangitis), secretion of a copious thin bile facilitates the flushing of the ducts [1913]. For this and for nonsurgical removal of hepatic stones [264, 3187] intravenous admin-

istration of sodium dehydrocholate has been recommended [1913]. A copious bile flow facilitates contraction of the gallbladder and prevents stone formation by keeping the bladder bile thin and alkaline, as evidenced by studies in experimental animals with biliary fistulas. Although choleresis flushes only the bile ducts and not the gallbladder, the frequent contractions of the gallbladder which occur secondarily appear to remove sediment. Bile acids have also been recommended in biliary dyskinesia, for the copious flow of bile seems to improve this condition.

Animal experiments indicate that the prolonged use of bile acids is not harmful [239]. The administration of bile acids to dogs with obstructed common ducts neither prolonged nor shortened their lives [1603]. The question whether bile acid administration is harmful in hepatic damage or in complete biliary obstruction is not settled. Recovery from arsphenamine jaundice is claimed to be accelerated by bile acids [86].

DYE EXCRETION

In the middle of the nineteenth century, the ability of the liver to excrete dyes into the bile was reported. Originally, this was considered to be a primary function of the Kupffer cells as part of the reticuloendothelial system [2303]. While this holds true for some dyes, such as trypan blue [3162], fluorescence microscopy has indicated that rose bengal [2264], fluorescein [1241], and azorubin-S [3613] are taken up primarily by the hepatic cells and do not pass through the Kupffer cells. The same can be assumed for Bromsulphalein [2265].

Histology of Dye Excretion. The liver has been observed after administration of dyes by vital microscopy and by the study of tissue sections. In frogs, fluorescein excreted in the bile completely outlines the bile canaliculi. Fluorescence microscopy in amphibians and mammals indicates that the dye rapidly enters the hepatic cells from the plasma and is transmitted without delay to the bile canaliculi [1066, 1241]. A refractory period exists, the peak of excretion occurs within an hour, and all excretion is complete within 24 hours. The bile canaliculi fill in an irregular fashion and show sprouting phenomena [1502] as a result of hepatic injury due to prolonged exposure during the period of observation [136]. In histologic sections, the regular polygonal canalicular meshwork becomes readily apparent after application of certain

dyes. Intracellular ramifications probably do not exist. Dyes such as rose bengal, injected intravenously, are taken up by the hepatic cells [2264] and rapidly cleared from the normal liver tissue, while in liver damage this action is delayed [3613].

Dynamics of Dye Excretion. The excretion of a relatively inert substance by the liver into the bile, the basic principle of dye excretion, is dependent upon several factors.

CHEMICAL NATURE OF DYE. The chemical structure, electropotential, and molecular size influence the excretion of a dye in the bile. In general, basic dyes and larger colloidal substances of low diffusibility are less readily excreted. The efficiency of excretion is greater in warm-blooded animals than in cold-blooded animals, and greater in carnivores than in herbivores [2245]. There is competition for excretion of dyes. For instance, rose bengal blocks the excretion of Bromsulphalein [2265].

ROLE OF KIDNEY. Most dyes are potentially excreted by the kidney as well as by the liver. Addition or removal of certain radicals determines the predominant pathway of excretion [2245]. Some substances are almost entirely excreted by the liver and others by the kidney. If either the kidney or liver is damaged, however, the other organ takes over at least part of the excretory function [895]. The urinary excretion of Bromsulphalein is negligible normally, whereas in liver damage it is variable and may be a possible source of error [1587, 2454]. If the dye is injected intravenously, more is excreted in the urine than if it is given orally [1085]. Phenolsulfonphthalein (PSP) is an example of a related dye whose pathway of excretion is predominantly renal and only slightly hepatic.

METABOLIC INFLUENCES. In hepatectomized animals up to 6 per cent of injected Bromsulphalein is disposed of by other organs [613]. This is not significant enough to disturb any calculations. Comparison between serum, hepatic, and biliary Bromsulphalein content during continuous intravenous infusion indicates a discrepancy of over 20 per cent, suggesting extrahepatic uptake or hepatic destruction [382]. In experimental biliary obstruction in dogs, pathways of Bromsulphalein removal other than biliary excretion exist. This is not true, however, in man [1163]. According to recent investigations with radioactive Bromsulphalein, this dye is metabolized to a certain degree in the liver [382]. Bromsulphalein excreted in the bile is absorbed in the intestine and therefore has

an enterohepatic circulation [2063], although this has been considered insignificant [2064].

CIRCULATORY INFLUENCES. Alteration of the circulation through the hepatic lobule influences the transport of the dyes to the hepatic cells, as well as the mixing time, and changes dye excretion. In heart failure, the Bromsulphalein excretion is impaired out of proportion to the degree of hepatic failure [321, 482, 1649]. Exact measurement of this influence became possible when the disappearance rate of Bromsulphalein from the blood was found to be fairly constant in man [1587]. In some hepatic diseases this disappearance rate decreases rapidly, suggesting hepatic saturation owing to decreased hepatocellular capacity. In other conditions, the disappearance rate is slow but does not reveal the saturation phenomenon, because of circulatory impairment involving either the liver as a whole or the lobular parenchyma [2265]. In the individual patient or under unusual experimental conditions such as hypothermia [411] this decrease can be the result of either hepatocellular or circulatory inadequacy. The reduced Bromsulphalein clearance in fatty livers, known for many years [1225], has been associated with interference with the sinusoidal blood flow by the enlarged fat-containing hepatic cells [1497, 1820], rather than with hepatocellular dysfunction. The importance of circulation in the clearance of Bromsulphalein from the blood by the hepatic cells is reflected in the use of the Bromsulphalein extraction by the liver in the technique for estimating hepatic or splanchnic blood flow (see Methods of Measurement, under Total Hepatic Blood Flow, Chap. 18).

ROLE OF HEPATIC FUNCTION. The removal of dyes by the liver has been widely used in hepatic function tests, the chromodiagnostic tests utilizing Bromsulphalein, rose bengal, and azorubin-S, as well as in x-ray visualization of the gallbladder and bile ducts. Many dyes, such as phenolphthalein, can theoretically be included. The delayed clearance of rose bengal from the liver in liver damage is the morphologic indication of the saturation of the hepatic cell. This was postulated by functional studies [2265], some using radioactive rose bengal [3296], in spite of the fact that the damaged hepatic cell tends more readily to take up dyes intravitaly [3613]. Comparison between serum clearance and biliary excretion also points to considerable storage in the liver for prolonged periods of time [382, 482].

Many factors related to disturbed hepatic function influence dye excretion. The presence of an Eck fistula reduces the rose bengal clearance. The lowest values are usually not obtained, however, until some time after the operation, indicating that secondary hepatocellular factors, rather than primary vascular ones, are of importance in this phenomenon [1088]. The reduction of Bromsulphalein excretion in external and internal bile fistula dogs is probably also the result of a disturbance of hepatocellular function [835]. During choleresis produced by administration of bile salts, the biliary excretion of Bromsulphalein is reduced, apparently as result of competition between bile formation and dye excretion [482, 2265]. Decreased excretion of Bromsulphalein is not necessarily accompanied by decrease in pigment output or even in the volume of bile.

FUNCTION OF THE NUCLEUS OF THE HEPATIC CELL

The nucleus is concerned with cell division and also represents an important regulating center of protein synthesis [368]. Desoxypentose nucleic acids of the chromatin, which are necessary for reproduction of genes, undergo changes in arrangement during the mitotic cycle in any cell [368]. Nuclear pentose nucleic acids control the nucleic acid metabolism of the other cellular structures, which are not concerned with the development of the self-reproducing gene. Impulses for the formation of protein flow from the nucleus through the nucleolus-associated chromatin, the nucleolus, and the nuclear membrane and are directed to the pentose nucleic acids of the cytoplasm (see Cytoplasm, Chap. 3) (Fig. 7). Growth and regeneration of the hepatic cells are thus under the influence of nuclear function. The nucleus of the hepatic cell, as in any other cell, basically has two functions; reproduction and regulation of growth. Growth has four aspects: embryonal and postnatal growth, adult growth or growth replacement, regenerative growth, and tumorous growth. Embryonal growth is discussed under embryology of the liver (see Embryology, Chap. 20).

of cytoplasmic pentose nucleic acid and an increased amount of cytoplasm, in addition to increased numbers of nuclei.

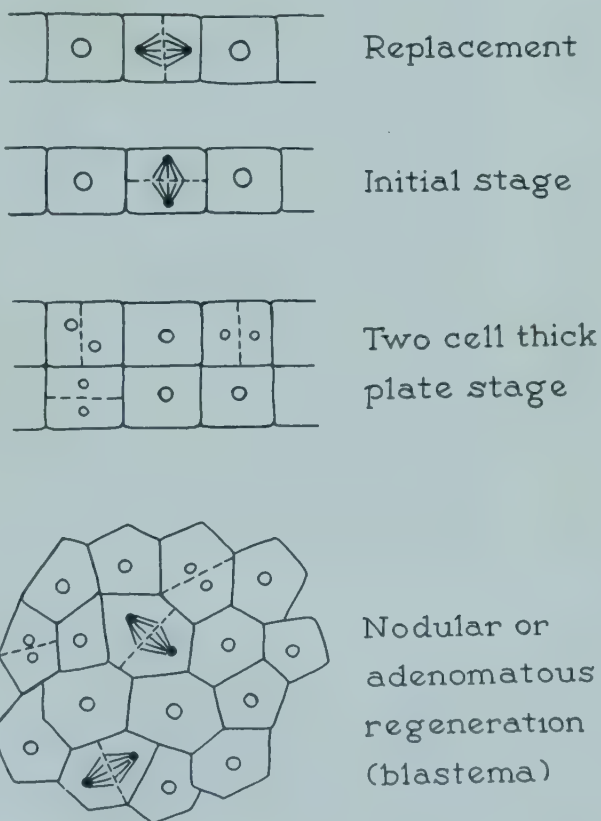


FIG. 29 Replacement growth and stages of regeneration of hepatic cells.

Whether mitosis is necessary for the cell division for normal replacement is debated [113]. Mitotic activity is normally present in the liver of weanling rats when the lobules enlarge into compound lobules [2140]. This activity can be suppressed by a low-protein diet, while a change to a high-

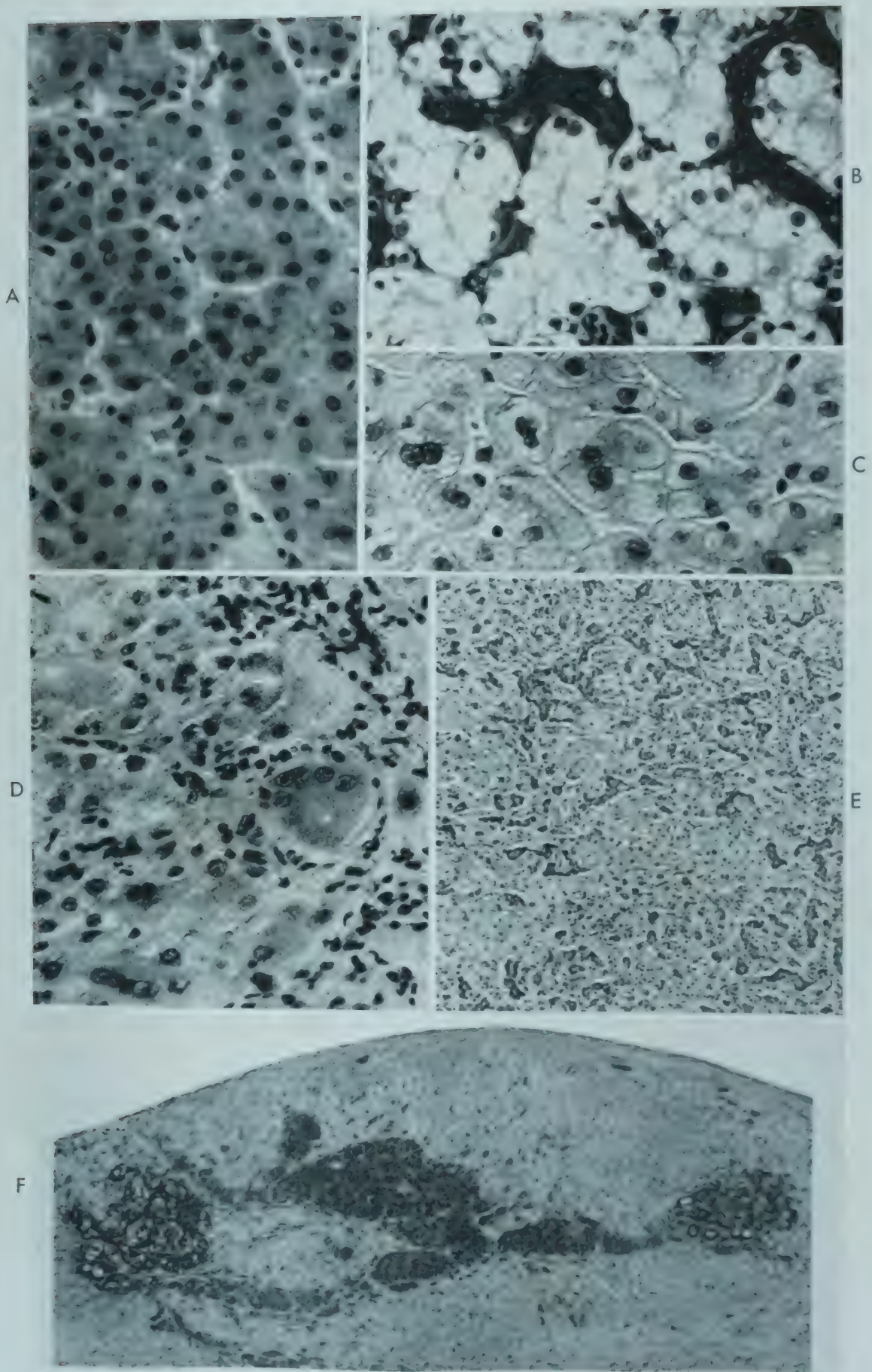


FIG. 30. Various types of regeneration, H&E. A, two-cell-thick plates in postnecrotic cirrhosis ($\times 230$). B, two to several-cell-thick plates in salamander livers ($\times 125$). C, binucleated cells in recovery from viral hepatitis ($\times 315$). D, giant-cell formation in subacute viral hepatitis; cellular condensation surrounding degenerating and regenerating hepatic-cell plates ($\times 275$). E, proliferation of ductules around hepatic cell remnants.

protein diet increases mitotic activity [1928]. In the adult mammalian liver, mitotic figures are rare or almost absent [746], although the normal nuclear picture is varied. For instance, two mature nuclei may be noted in a single cell, sometimes connected by a bridge; this has led to the assumption of an amitotic division. The different sizes of nuclei represented by the geometric series, 1:2:4:8, have also been considered as evidence of amitosis, the nucleus supposedly dividing when its volume doubles [153]. Amitotic division has been claimed to only enlarge the nuclear surface without cell division [2583]. Recent studies [3623] have suggested, however, that binucleated cells result from mitotic divisions with failure of the cytoplasm to divide. One of the nuclei may become small and pyknotic. Secondary fusion of the nuclei is also possible, producing multipolar and polyploid nuclei; cells may even fuse and separate again. In all these processes, the nuclear mass does not actually grow. The common nuclear variations therefore are not necessarily an indication of amitosis, and both mitotic and amitotic cell divisions probably occur in the normal liver, the former predominating during accelerated growth.

Stimuli for Mitosis. Mitotic activity may be increased without recognizable loss of cells. Repeated injections of colchicine, for instance, lead to numerous mitotic figures as a result of arrest of mitosis [2314]. Liver and kidney tissue extracts, boiled egg yolk, and trypan blue increase mitoses, possibly by acting as growth factors (see Regeneration, later in this chapter). Slight alteration of cells may stimulate mitosis, as in experiments with Coramine, which produces hydropic cells with intact basophilia [3623]. The greatest impulse for mitosis is produced by loss, necrosis, or damage of the cells. Abnormalities of mitoses are associated with variations of nucleic acid concentrations.

Tissue Culture of Liver. Parenchymal hepatic cells are more difficult to grow in tissue cultures than most other cells in the liver and particularly more difficult than the bile duct cells. If the growth of these other cells is suppressed, pure cultures of parenchymal cells can be obtained [811]. Cultures develop better from embryonal

tissue, and even human fetal tissue has been successfully cultured [2582]. Mitoses or metabolic processes such as glycogen and pigment deposition have been studied. Adult tissue can be cultured only in the presence of embryonal extracts, which are necessary, for instance, for the production of lipofuscin in cultures. Liver pieces were transplanted into the anterior chamber of the eye [327] or under the skin; little proliferation of hepatic cells and more bile duct proliferation resulted [1470] (Fig. 30F).

REGENERATION

The replacement of cells lost in normal wear and tear is actually a type of regeneration but has been discussed under adult growth. Regeneration, in a stricter sense, results from damage, necrosis, or removal of liver tissue. The classic way to study regeneration is partial hepatectomy, which rapidly produces changes in the remaining liver. Damage to hepatic cells in liver disease or damage produced by hepatotoxic agents or bile duct ligation also stimulates regeneration. The degree and character of the regeneration, a fundamental process in hepatic injury, are important in the structural and functional picture of various liver diseases.

The regenerative potentialities of the liver were first described in 1833 by Cruveilhier. If 75 per cent of the liver is removed, complete restitution occurs within 8 weeks in dogs and within 3 weeks in rats [1484]. In animals with multilobulated livers, different lobes can be successively extirpated so that the total amount removed may eventually far exceed the original size of the liver [2202]. The capacity for growth is also seen in the plasticity of the organ, which permits it to be molded by pressure exerted by ribs, corsets, or tumor masses. New lobules are said to be formed in hypertrophy, but this claim is not fully accepted [2202].

Morphologic Appearance of Regeneration

The classic analysis of Herxheimer and Thölldte [1470] presents the earlier literature on the morphology of degeneration. Mitotic activity seems to

in diffuse septal (alcoholic) cirrhosis ($\times 70$). F, four-week-old transplant of liver in anterior chamber of eye in a rabbit. Bile ductal and ductular proliferations, as well as accumulations of pigment-containing macrophages, are surrounded by fibrous tissue ($\times 33$). (Boeck, J., and Popper, H.: *Virchows Arch. f. Path. Anat.* 299:219, 1937; D and E, Popper, H.: *Am.J.Med.* 16:98, 1954.)

accompany all types of regeneration, in contrast to normal replacement which may be amitotic. Depending upon the extent and site of injury, five types of responses occur:

1. The response to the disappearance of scattered cells, not clearly differentiated from normal replacement
2. The response to the disappearance of part of the lobule
3. The response to the disappearance of a small part of the liver consisting of several lobules
4. The response to actual or functional removal of a large part of the liver
5. Nodular regeneration

Intralobular Regeneration in Intact Lobules.

This is associated with various anatomical changes, such as chronic passive congestion, fatty infiltration, and senile or starvation atrophy [113]. It may occur, however, without obvious morphologic alterations, especially in older people. It differs from simple replacement by reverting to embryonal growth patterns. The replacement of normal wear and tear occurs within the plane of the original, one-cell-thick, hepatic-cell plate, with the plane of division between the newly formed cells perpendicular to the plane of the plate. In regeneration the plate widens, in addition to possibly lengthening, so that division occurs also in a plane parallel to the plane of the plate, resulting in the development of two-cell-thick plates (Figs. 29 and 30A). Occasionally they may become several cells in thickness and thus form a blastema resembling the embryonal liver [907] or the liver of lower vertebrates [908] (Figs. 29, 30B, and 31, upper right). The nuclei are greatly enlarged with increased chromatin [3262] and are occasionally irregular in shape but show no chromosomal irregularity [274] (Fig. 30C). The nucleoli become large and may increase to five or six in number. Multinucleated and polypoid cells are common [190] (Fig. 30D). In early active regeneration, mitosis predominates, whereas in slower stages binucleated and multinucleated cells, which are often considered results of amitotic division, are in the foreground [586]. Eosinophilic nuclear inclusions are commonly noted [73]. The cytoplasm is densely basophilic, although it is sometimes rich in glycogen [113]. Fatty metamorphosis occasionally occurs in regeneration, but it may also stimulate regeneration. Experimentally, diffuse regeneration in intact lobules can be produced by feeding ethionine, a biologic antagonist of methionine. While hepatic cells degenerate, bizarre re-

generation of neighboring hepatic cells results in the formation of blastemas several cells thick and in widespread intralobular proliferation of ductules [2635]. An analogous lesion in the human liver occurs in viral hepatitis, where degeneration coexists with regeneration.

Regeneration and Replacement of Partially Destroyed Lobules. The morphologic picture of regeneration after partial necrosis of a lobule resembles regeneration in intact lobules. It is seen in necrosis caused by viral infections, by toxic substances, or by vascular interference, some of which conditions have been produced experimentally [3571]. After removal of the necrotic debris, hepatic-cell plates, usually two or more cells thick, grow in the preformed spaces delineated by the intact framework. If much of the lobule is destroyed, the framework partially collapses (see Submassive Collapse, under Forms of Fibrosis, Chap. 27). The remaining hepatic cells develop into separate nodules with different degrees of regeneration. In the collapsed tissue many ductules are noted (Fig. 31, bottom).

The regenerating cells may differ from normal hepatic cells. The regenerated hepatic cells, particularly in children, may be extremely bizarre, with formation of syncytiums, with or without giant cells, which may assume unusual shapes and enlarge (Fig. 31, upper left). Since no cell membrane forms, no bile canaliculi develop, and therefore bile pigment vacuoles accumulate in the cytoplasm of these cells. Normally developed neighboring cells may contain no bile pigment. These giant cells have also been considered to be malformations [3094]. Another form of regeneration with very flat epithelial cells has been considered especially resistant to additional injury and is supposedly common in older animals [2159].

Sometimes the arrangement of cells closely simulates that of proliferating bile ductules (Fig. 30E). The morphogenesis of such structures has been widely debated. Bile ductules proliferate, especially if the parenchymal lesion is located near a portal tract, resulting in severance of the connections between ductules and bile ducts (see Ductular Proliferations, under Regeneration of Bile Ductules and Ducts, Chap. 16). Embryologic evidence (see Bile Duct Development, under Embryology, Chap. 20) indicates that many such structures are probably derived from hepatic cells rather than from bile ducts. Nevertheless, most authors refer to these changes as proliferating bile

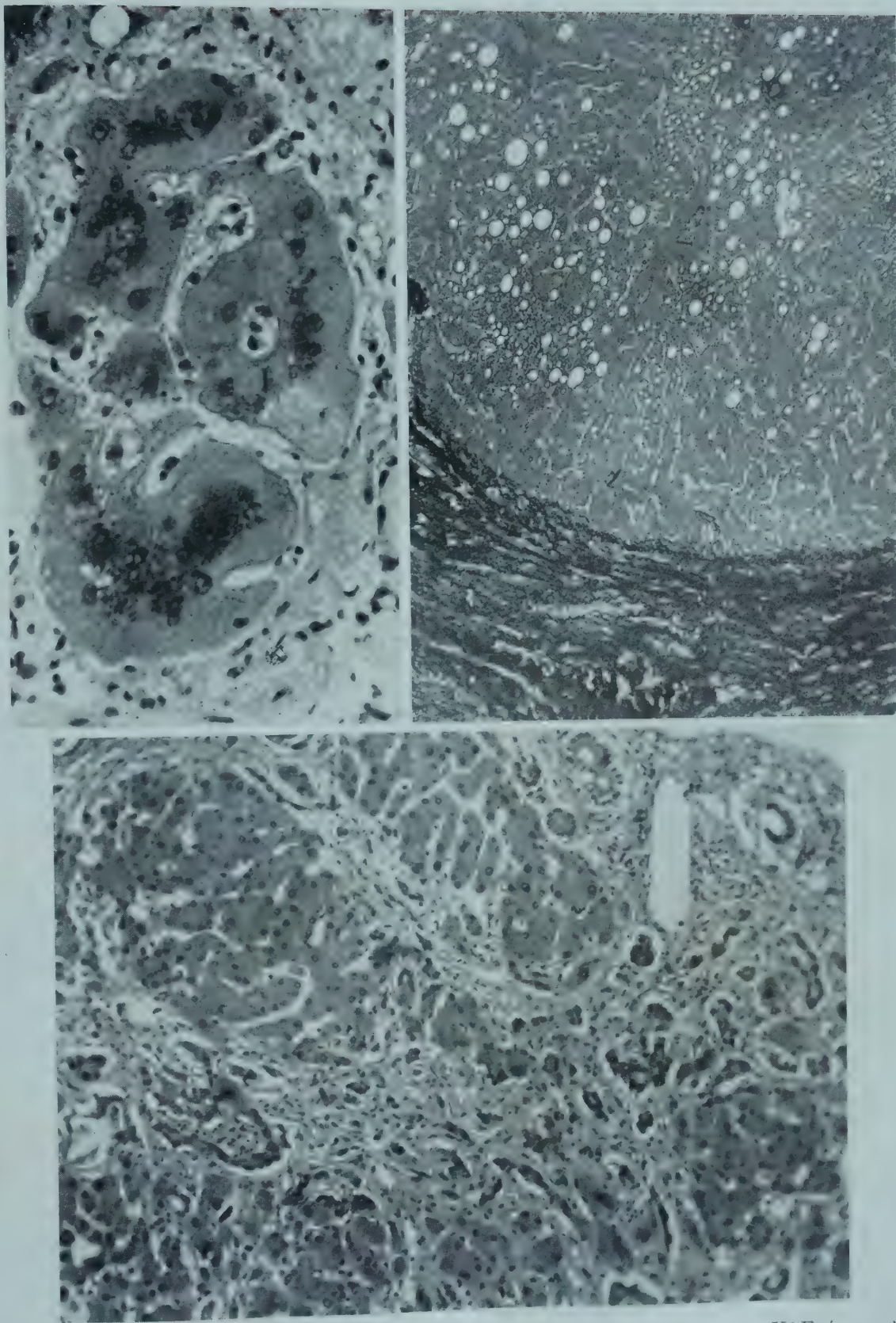


FIG. 31 *Upper left.* Syncytial multinucleated giant cells in infantile hepatitis. H&E ($\times 230$). *Upper right.* Regenerative nodule consisting of several-cell-thick plates on its periphery in postnecrotic cirrhosis. Mallory aniline blue stain ($\times 55$). *Bottom.* Submassive collapse with irregular regeneration of cells in nodules trapped in connective tissue. In the collapsed connective tissue many ductules are noted. H&E ($\times 120$).

ducts or ductules, and therefore the details of the lesion and the argument concerning its morphogenesis are presented in the section on regeneration of ductules (see Regeneration of Bile Ductules and Ducts, Chap. 16).

Regeneration after Necrosis of Groups of Lobules. Destruction of entire lobules limits the regenerative ability of the hepatic cells, and a fibrous scar develops, either as a result of a massive necrosis of all hepatic cells of a lobule, with subsequent collapse of the framework (Fig. 93, lower, Chap. 22), or as a result of traumatic or surgical removal of a small part of the liver. In this scar, ductular structures are abundant.

Regeneration after Removal of a Large Part of the Liver. Following partial hepatectomy, the picture described above is seen at the removal site [116, 3678]. Significant changes occur in the remote areas of the remaining liver. The recovery period can be divided into two stages. The first, rapid restoration of hepatic cells, is usually complete after 1 week. The second is a more gradual regeneration, often associated with focal necrosis, which lasts about 1 month [3624, 3677]. Entire lobules can regenerate, following the pattern of embryonal growth. The changes in the first phase include enlargement and proliferation of individual cells [421]. Mitoses are seen very early, primarily near the portal tracts [1384], whereas cytoplasmic division lags, resulting in binucleated cells. There is an altered staining of the framework, which has been considered an expression of depolymerization [116]. The regenerating cells are at least temporarily rich in pentose nucleic acids and lipids and are often poor in glycogen [3678]. The cytoplasm, nuclei, and nucleoli enlarge on the first day in regenerating rat liver. When cell division begins, this becomes less apparent [3239]. The cellular nucleic acid concentration, as measured by ultraviolet microscopy, appears increased in the nucleus and perinuclear cytoplasm when cell division becomes prominent. Alkaline phosphatase increases around the regenerating bile canaliculi [3678]. Extravascular spaces and sinusoids are relatively decreased by the expanding parenchymal cells [1384]. In this type of regeneration the hepatic-cell plates become two and more cells in thickness, reverting to an embryonal appearance and blastema formation. If this process is exaggerated, nodules are produced without polarity of their blood flow. Such hyperplastic nodules are not easily differentiated from adenomas [1470] (Fig. 31, upper right). This remote

regeneration is stimulated by a humoral mechanism, as shown in parabiotic rats. The liver that has not been operated on is stimulated by partial hepatectomy of the parabiotic partner, as measured by increased liver weight and increased mitosis [430, 578, 3558]. The same humoral effect is postulated if large parts of individual lobules or several whole lobules in different parts of the liver are destroyed by disease processes or intoxications. This explains the excessive adenomalike regeneration often encountered. The humoral factor exerts its influence even on fibroblasts in tissue cultures [1191].

Nodular Regeneration. If isolated groups of cells or even single cells are separated from the remaining lobular parenchyma, they often proliferate, stimulated by the reduction of liver tissue that occurs as part of the disease process. They form groups of hepatic cells with newly formed reticulum fibers and sinusoids. The latter develop by extension from neighboring canaliculi or sinusoids. These newly formed sinusoids converge to a newly formed efferent vein in the center of the nodules. Occasionally complete portal tracts reform, in addition to intraparenchymal connective tissue and efferent veins. Regenerative nodules are an integral part of cirrhosis formation (see Formation of Regenerative Nodules, under Processes Common to All Types of Cirrhosis, Chap. 28).

Chemical Processes Associated with Regeneration

Chemically, a conspicuous change occurs in the nucleic acids in the regenerating liver. Desoxypentose nucleic acid, as expressed per nucleus, increases rapidly in the first 24 hours before cell division begins [2668]. The rate of formation of DNA is approximately double that of cell formation, indicating that mitosis is associated not only with formation of additional DNA but also with replacement of the original DNA [3217]. The pentose nucleic acid of the cytoplasm is not increased so much, and the protein nitrogen level is slightly elevated, especially in the microsome and supernatant fractions [823, 1554, 2462, 3368, 3624]. The incorporation of water, glycogen, lipid, and protein into the regenerating liver does not occur at equal rates. The incorporation of lipids and proteins starts on the first day, while that of glycogen begins on the second day [1307]. The soluble liver proteins are usually normal [3137]. Tissue amino acids and glutathione [580, 998], cytochrome C [823], and other enzymes [2828], including phosphatase [430, 2827, 3368], are in-

creased. Phospholipids are increased [3367], but their turnover is depressed [551]. In contrast to what happens in neoplastic tissue, anaerobic glycolysis is not increased [2455]. The collagen content of the liver decreases during regeneration [1384]. Chemical patterns during regeneration suggest that the submicroscopic particles containing protein, phospholipids, and pentose nucleic acids increase more rapidly than the mitochondria, since the succinoxidase activity characteristic of the mitochondria is reduced initially [1384].

Factors Influencing Regeneration

DIET. A mixed high-carbohydrate and high-protein diet enhances regeneration, especially in hepatic injury, but a balanced diet in general is superior to one weighted in any direction. Protein is initially more rapidly deposited in the liver of the protein-starved animal, although eventually the protein-fed animal deposits much more [1307]. Administration of moderate amounts of fat does not interfere with regeneration [2799]. Powdered whole liver increases the rate of regeneration beyond the effect of its high protein content [2434], possibly owing to its nucleic acid content. The addition of PNA or DNA to the diet accelerates regeneration [2433], perhaps by eliminating the necessity of nucleic acid formation [2433]. Vitamin B₁₂ also seems to enhance regeneration [861].

CIRCULATION. Circulation is important in regeneration, and one of the main functions of regeneration is the reestablishment of normal circulation [2202]. The portal blood flow is said to be of major importance in regeneration. Birds with a natural portosystemic anastomosis show poor regeneration after partial hepatectomy [2219]. In the presence of Eck fistulas, regeneration stops in the anatomically undistorted liver [1284, 2202]. A side-to-side portocaval shunt reduces the blood flow through the portal vein but not so much as an Eck fistula, and regeneration following partial hepatectomy occurs but at a slower than normal rate [2202]. In dogs with portocaval anastomoses, complete regeneration occurs if the inferior vena cava is constricted above the liver, thus raising the intrahepatic intravascular pressure [1284]. This observation led to the conclusion that the volume of portal blood and its pressure determine the rate of regeneration [567, 1284]. If the portal vein and inferior vena cava are transposed, however, regeneration following hepatectomy averages only 50 per cent of normal, indicating that portal

vein blood provides specific additional stimulation [565].

Hepatic vein obstruction curtails regeneration somewhat. The hepatic artery is of less importance [1695]. Anoxia itself seems to be without influence [823]. Forced exercise has no effect on regeneration [2259]. In the cirrhotic liver the degree of restoration is determined by the intactness of the hepatic blood flow and, specifically, by the portal venous flow.

HORMONES. Increased regeneration following administration of thyroid extract was probably the first known hormonal influence upon hepatic regeneration [486, 943, 1484]. Increased regeneration also occurs, however, upon thiouracil administration [693], while neither thyroidectomy [823] nor hypophysectomy strikingly interferes with regeneration. The nature of the thyroid effect is, therefore, not established. Adrenalectomy interferes with regeneration, while desoxycorticosterone, as well as other corticosteroids, stimulates it [1096].

AGE. Young rats exhibit a far greater regenerative ability than adult or old rats [430, 2455]. The interval between hepatectomy and the peak increase in mitosis is delayed in old rats [2223].

OTHER FACTORS. Obstruction of the biliary flow reduces or completely abolishes regeneration of parenchymal cells [1484, 2202]. Many other factors seem to influence hepatic regeneration, such as vitamins [1841], splenectomy [1484], and the presence of fibroblasts (in tissue cultures) [811].

REGULATING MECHANISM. The facts presented fail to explain why the liver regenerates rapidly after loss or injury, or what the regulating mechanism is that causes the liver to stop regenerating after it regains its normal size. The liver undoubtedly contains growth factors for tissues in general, an early observation now more significant in view of present knowledge of growth factors of the vitamin B₁₂ and folic acid series. The parabiosis experiments point to a humoral factor reminiscent of the old "wound hormone," known to exist in plant tissues. Anoxia, indicated by the vacuolization of the hepatic cells, is thought to cause depolymerization of the ground substance and loosening of the connective tissue framework, permitting expansion of the remaining cells by cell division [631]. The concept of functional demands determining regeneration has been challenged. Blood flow under increased pressure through the hepatic sinusoids has been considered the chief stimulus [2202].

According to recent studies confirming Hering's original concept, the hepatic plates surround a communicating system of lacunae, or cavities, that are much taller than they are wide [907]. These pervade the entire lobe as the "hepatic labyrinth." A sacculosinusoidal type of liver in man, in which the lacunae are wide and irregularly shaped, can be differentiated from a tubulosinusoidal type in the rabbit and horse, in which the lacunae are narrow and almost cylindrical. The lacunae are spaces in which the sinusoids are suspended. The sinusoids are lined by endothelial cells on a basement membrane, as in other capillaries. However, several structural and functional characteristics differentiate them from capillaries elsewhere and justify the term "sinusoids."

STRUCTURAL CHARACTERISTICS OF THE HEPATIC SINUSOIDS

In contrast to the otherwise uniform capillaries throughout the body, sinusoids vary in width from 4 to 15 μ .

Lining of Sinusoids. The lining of the sinusoids merges with the intralobular connective tissue framework, which appears as a fine fibrillar structure in routine histologic sections. It connects the sinusoids with the hepatic-cell plate, which has no basement membrane of its own, in contrast to other glandular structures (Fig. 32A). The basement membrane of the sinusoids consists of a network of reticulum fibers, best demonstrated with silver impregnation (Fig. 32B). They can also be seen with Mallory's aniline blue or trichrome stains but do not normally give Van Gieson's collagen reaction except near the portal or central fields, where some collagen fibers may be present to serve as anchors for the other fibers. The larger

and thicker fibers, some of them probably membranes, are parallel with the long axes of the sinusoids. The finer and shorter fibers or smaller membranes are arranged in a lacelike fashion. The latter traverse the perisinusoidal space, buttressing the sinusoidal wall against the hepatic-cell plate. On the outer surface a few perithelial cells, pericytes, with spindle-shaped nuclei and no fibroblasts can be seen [2582].

The histochemical nature of the intralobular connective tissue that includes the sinusoidal lining is not well known. In routine sections it does not give a periodic acid reaction normally [1924]. In dry-frozen preparations a fibrillar basement membrane has been demonstrated. It assumes a sheet-like form in regeneration, suggesting depolymerization of polysaccharides, which would permit greater plasticity of the framework [116]. With allochrome stains only the blue color of collagen-like structures can be demonstrated. With a modified iron and ammonium aluminum sulfate cochineal technique, no ground substance can be stained [2773]. In experimental cirrhosis an interfibrillary ground substance has been demonstrated with the periodic acid routine [145].

Kupffer Cells. The inner surface of the sinusoids is formed by endothelial cells. In contrast to the situation in other capillaries, cell borders can not be visualized by the usual silver impregnation methods. It is, therefore, not established whether or not the thin cellular lining is continuous. The endothelial cells differ from those of other capillaries by their great variation in size and shape. Some are flat, undifferentiated cells with small nuclei and dense chromatin, like other endothelial cells. Others have large vesicular nuclei and prominent nucleoli and bulge into the lumen in a wing-shaped fashion (Figs. 33, 34, upper left). Rav-

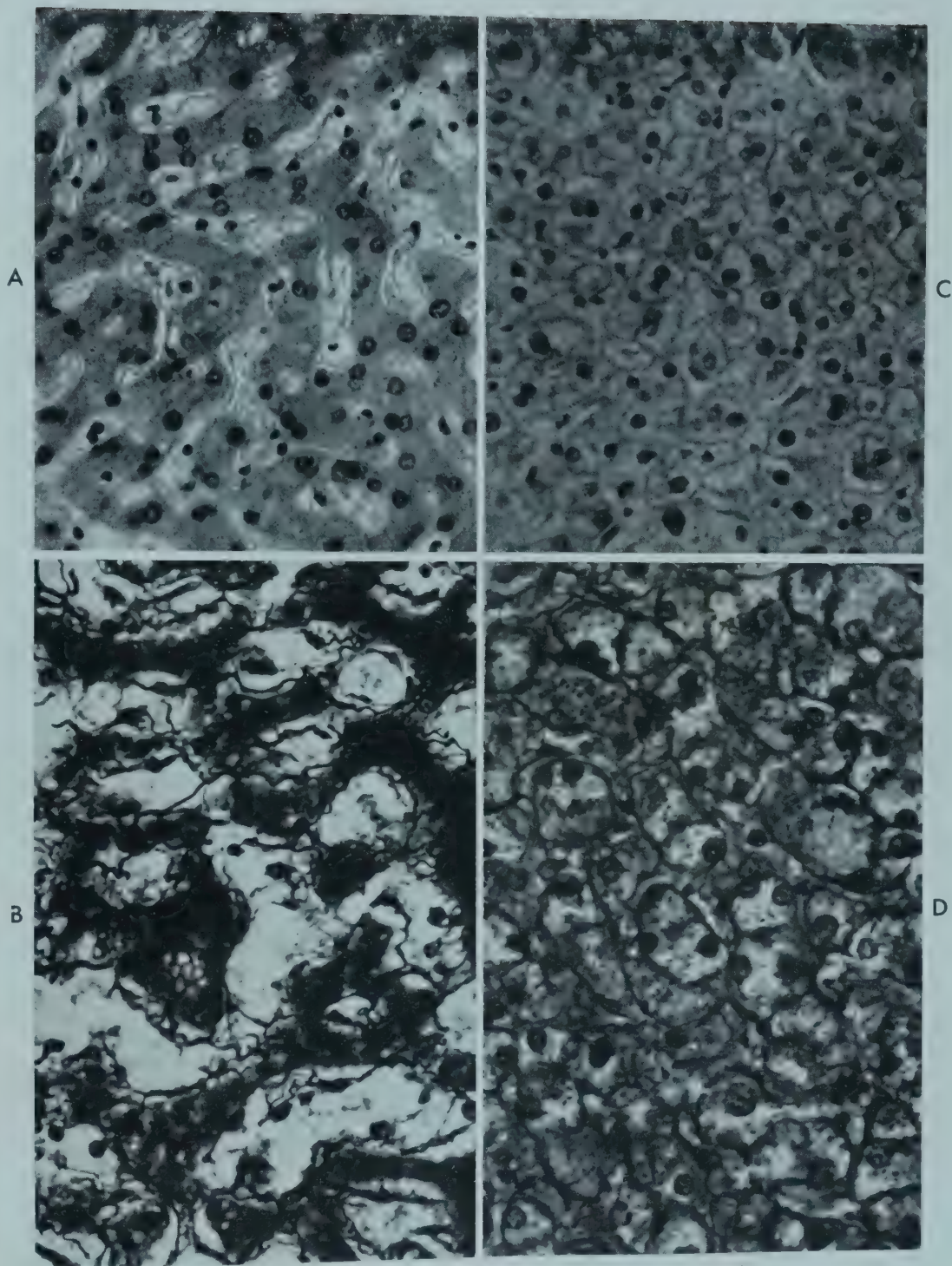


FIG. 32 A, appearance of the liver after death from head injury following a short agonal period. B, appearance of the liver in hepatic edema caused by nephrosis. The perisinusoidal spaces in A and B are dilated, and the expanded reticulum framework is represented by split axial fibers and cross fibers traversing the wide perisinusoidal spaces. C and D, appearance of the liver in instantaneous death caused by an airplane crash. The perisinusoidal spaces are not visible, and in silver stains the basement membrane of the sinusoid is represented by one line. A and C, H&E ($\times 240$); B and D, silver impregnation of reticulum fibers ($\times 475$). (B and D, Popper, H.: *Arch. Path.* 46:132, 1948.)

like extensions from these cells gave them the name of "stellate cells" of von Kupffer. Some appear entirely detached from the sinusoidal lining and seem to float freely in the lumen, an illusion due to the two-dimensional nature of the section [907] (Fig. 33); under abnormal conditions, however, these cells may be discharged into the blood, so that transitional stages are probable. Protoplasmic projections of the Kupffer cells between the cells of the hepatic-cell plates toward the bile canaliculi may be artefacts or a pathway for the direct discharge of bile pigment from the Kupffer cells to the bile canaliculi [2544].

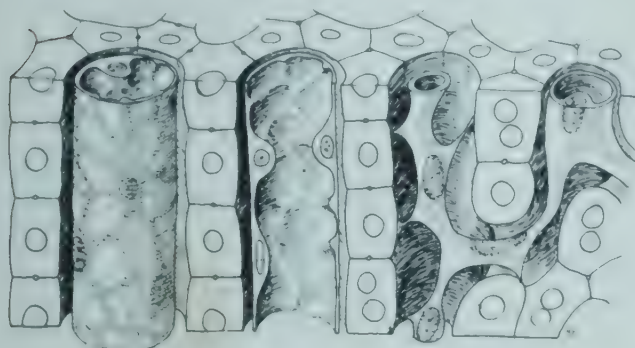


FIG. 33 Three-dimensional drawing of Kupffer cells and sinusoids surrounded by perisinusoidal spaces. (Elias, H.: *Chicago Med. School Quart.* 13:60, 1952.)

INCLUSIONS IN KUPFFER CELLS. The cytoplasm of the Kupffer cells may normally contain many granules or inclusions, especially under various abnormal circumstances, contributing to the pleomorphism of these cells. The inclusions are related to the metabolic and phagocytic activities of these cells; protein particles, lipids, iron pigment (Fig. 34, upper right), vitamin A (Fig. 34, lower left and lower right), and fragments of leukocytes, as well as PAS-positive material, may be demonstrated. In addition, some granules which can be stained with the periodic acid routine have been considered precursors of connective tissue or engulfed basement membranes [116]. Normally erythrocytes are not seen, but in many diseases fragmented or intact red cells are found within the Kupffer cells. A large variety of blood and bile pigments, as well as wear and tear pigments, can be demonstrated; their presence is not necessarily abnormal. Bile pigments are greatly increased in the various forms of jaundice, in blood pigment with or without demonstrable iron in various blood dyscrasias and after blood transfusion, and in wear and tear pigment in debilitating conditions.

Pigmented and nonpigmented exogenous sub-

stances are demonstrable in Kupffer cells, again being present in excessive amounts under abnormal circumstances. These substances include bacteria, pigments resulting from medication, breakdown pigments of such parasites as those of malaria or schistosomiasis, and the pigment discharged to the blood stream from lymph nodes, as in anthracosis or silicosis.

ENDOTHELIAL VS. KUPFFER CELLS. The difference between the flat endothelial cells and the Kupffer cells is more apparent after injection of dyes that are taken up only by the Kupffer cells. Even in routine histologic sections, transitions between the two types of cells can be noted. Vital microscopic studies on frog and monkey livers fail to show any differences between the cells [1808]. In the frog, the sinusoids have a continuous cellular lining and each of the lining cells can bulge forward into the lumen to become a Kupffer cell, although such cells lack the pseudopodial processes seen in fixed specimens. The so-called "stellate cell," therefore, is the functionally active stage of the hepatic sinusoidal endothelium.

FUNCTIONAL CHARACTERISTICS OF THE HEPATIC SINUSOIDS

The sinusoids are distinguished from other capillaries by different membrane function, or permeability, and by different cellular functions, the hepatic endothelial cells being active parts of the reticuloendothelial system, with special metabolic functions.

Specific Permeability

Originally tissue fluid was thought to be free of protein, but subsequently much protein was normally found to pass through the capillary membrane and appear in the lymph. The hepatic sinusoids are more readily permeable to serum proteins than other capillaries, as suggested by the high protein concentration in hepatic lymph. Its protein content rises following relatively slight hepatic injury; for instance, the protein content of the thoracic duct lymph is increased by anesthesia or other slight irritations [945]. In man, during the agonal period, the perisinusoidal spaces of Disse dilate rapidly, probably owing to anoxia, and appear filled with precipitated protein [2625] (Fig. 32). Three distinct permeability phases of the lining membrane can be seen during vital microscopy [1808]. In one phase, the distance between individual red cells passing through the

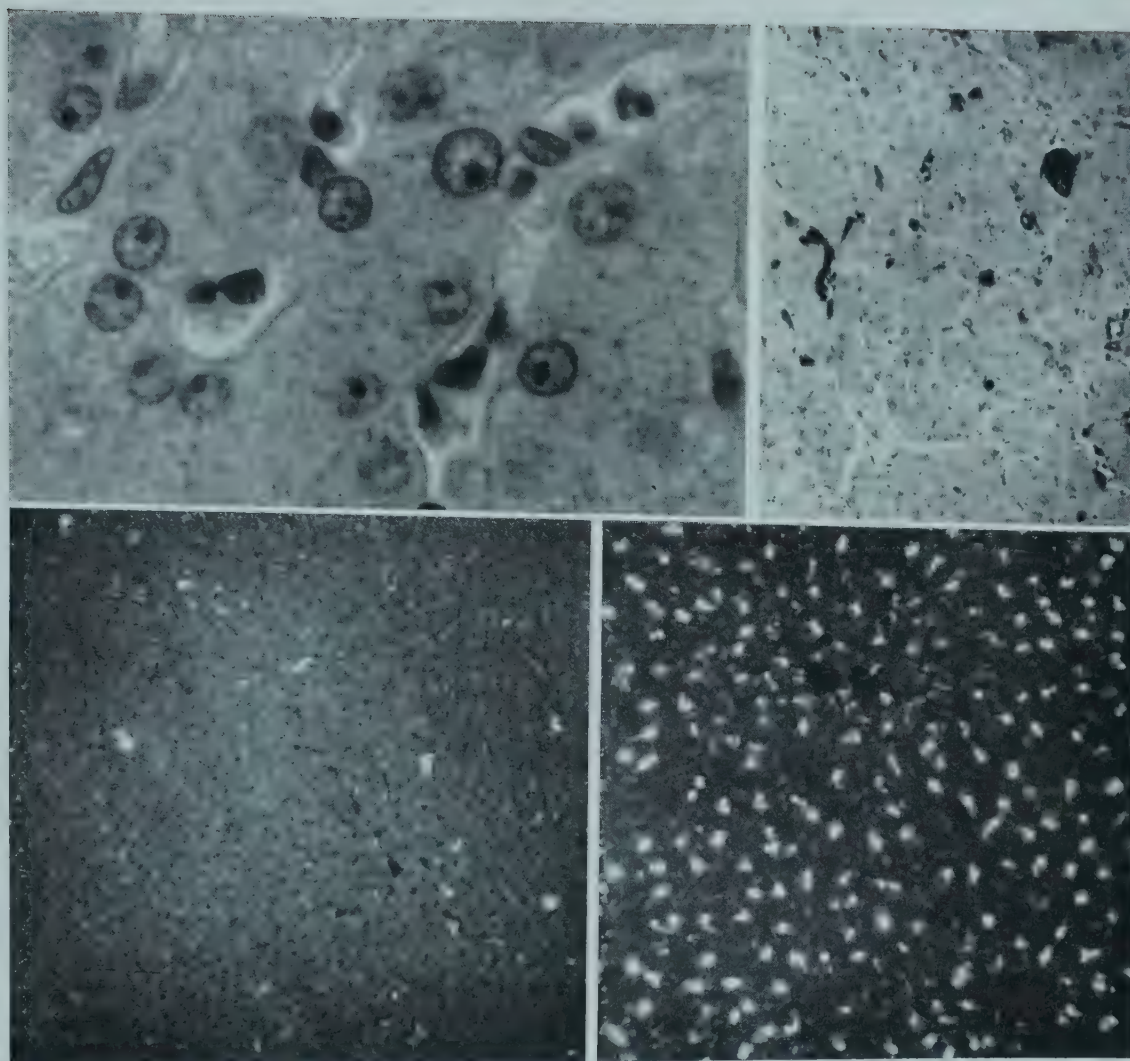


FIG. 34 *Upper left.* Proliferation of Kupffer cells, which bulge forward into the lumen of the sinusoids in subacute bacterial endocarditis. H&E ($\times 610$). *Upper right.* Iron pigment in Kupffer cells in hemosiderosis. H&E ($\times 120$). *Lower left.* Vitamin A fluorescence, mainly in Kupffer cells, in human liver ($\times 80$). *Lower right.* Strong vitamin A fluorescence of Kupffer cells in rats with hypervitaminosis A ($\times 80$). (C and D, Popper, H.: *J. Mt. Sinai Hosp.* 7:19, 1940.)

sinusoid is not altered, indicating that little, if any, fluid passes through the sinusoidal membrane. In another phase, the red cells become more closely packed as they approach the center of the lobule, indicating that much serum escapes into the perisinusoidal space. Under these conditions, the lymph and serum concentrations of protein are almost equal. An intermediary phase is indicated by a closer approximation of the red cells without packing. The great permeability of the hepatic capillaries for protein assists in the fluid and metabolic exchanges in the liver.

Kupffer Cells as Part of the Reticuloendothelial System

Acid azo dyes injected into animals are selectively accumulated in different sites, such as the Kupffer cells of the liver, reticulum and endo-

thelial cells of the spleen, endothelial cells of lymph nodes, bone marrow, adrenal and pituitary glands, and in reticulum cells of the loose areolar connective tissue, the resting wandering cells. Although these cells have different functions and appearances, their common phagocytic ability induced Aschoff [108] to coin the name "reticuloendothelial" system, implying that all elements capable of phagocytosis are related to each other, with the exception of the microphages of Metchnikoff, the neutrophilic leukocytes. The reticuloendothelial elements are considered to be derived from both connective tissue reticulum and endothelial cells [108]. These cells, some of which are called histiocytes, are related to the monocytes of the blood.

The more dye injected, the more sites of deposition are found, until almost all mesenchymal

cells are laden with it. The reticuloendothelial system has a systemic and a local portion. The systemic portion, in which the liver is of greatest importance, concerns phagocytosis of material circulating in the blood. The local portion is the mesenchyme of the various tissues, which removes material locally accumulated. Dye injections indicate that the Kupffer cells are not the only reticuloendothelial cells of the liver. Under normal circumstances a few histiocytic cells are found in the portal tracts, which have similar phagocytic ability. In inflammatory conditions and in cirrhosis the number of these cells may be considerably increased. The differentiation of these cells from plasma cells is often difficult.

A sharp borderline for the reticuloendothelial system can not be drawn, since almost any mesenchymal cell may assume the function specifically assigned to the system. As the demarcation of the reticuloendothelial system has become less distinct, more functions have been assigned to it. The retention of the name appears justified, to connote a first line of defense against generalized insults which may be supplemented by additional defenses if the attack either is overwhelming or hits one particular area. The Kupffer cells of the liver are in the main line of defense and share most of their functions with other reticuloendothelial cells. Different stages of function are indicated by the variations in size of the Kupffer cells, and, for instance, by storage of dyes in only a few cells at a time [802]. The functions of the reticuloendothelial cells in the liver also include antibody and globulin formation, blood breakdown, and bile formation, and possibly some metabolic functions.

General Phagocytosis. MATERIAL ENGULFED. Kupffer cells engulf various exogenous and endogenous substances. The endogenous substances are usually no longer usable or usable only after extensive structural changes. Phagocytosis accounts for the presence of the various endogenous pigments, some of which are best visualized by fluorescence microscopy [1172, 2633]. For experimental demonstration of phagocytosis, dyes, colloidal material such as india ink, and heavy metals have been used. The effect of india ink in some instances has been ascribed to some toxic factor present in it, rather than to phagocytosis of carbon particles [3716]. Colloidal iron is easily demonstrated by iron reactions [943]. Manganese dioxide is removed by the Kupffer cells. Recently, radioactive chromium uptake by the Kupffer cells

has been applied as a quantitative measure of Kupffer cell function [2707] and of hepatic blood flow. Thorium dioxide, which has been used for radiographic demonstrations of the liver and spleen in man, is stored for many years in the Kupffer cells [513]. Silica particles are also seen in the Kupffer cells if the particle size is small.

FACTORS IN PHAGOCYTOSIS. Some dyes are not so readily stored as others. Earlier publications indicated that primarily electronegative dyes are engulfed. Electropotential was considered the basis of phagocytosis, and the particle size was thought to be of secondary importance. The amount of dye injected is indicated by the number of cells showing phagocytosis. This number can be increased by repeated injections, showing that different stages of function are exhibited by various cells.

MECHANISM OF PHAGOCYTOSIS. Two processes are involved in phagocytosis: (1) contact of the material to be engulfed with the phagocyte; (2) the acceptance of the material by the phagocyte. Recent vital microscopic investigations have clarified why the Kupffer cells ingest some particles and not others [1808]. In frogs, injected particles of india ink or other foreign material are coated by a glossy precipitate, apparently protein in nature, derived from the plasma. This coating has streaming processes, which stick to the sinusoidal lining when they touch it. The coated foreign body is then taken into the cytoplasm of the Kupffer cell, which now bulges into the lumen, or perisinusoidal space. Only very small coated particles may pass through the liver, remaining in the axial stream. Particles without streamers do not readily touch the sinusoidal wall, but if they do, they may or may not be engulfed. For instance, uncoated red cells are not absorbed even if they are in close contact with the wall. The presence of the coating, therefore, determines the selectivity of phagocytosis, and stasis is not necessary [1808]. Adequate hepatic circulation, however, is important. Increased blood flow accelerates phagocytosis, while heparin prevents it by inhibiting the formation of the coating [1808]. Surface-active agents, such as bile acids and detergents, increase phagocytosis [1515].

Bacterial Phagocytosis. EFFICIENCY OF UPTAKE. Bacteria injected intravenously are demonstrable in the Kupffer cells and in other reticuloendothelial cells. The liver and spleen are the most efficient organs in bacterial phagocytosis. In hepatic vein blood of patients with subacute bacterial endocarditis, an average of 85 per cent ex-

traction has been found [204], in keeping with the histologic observation of proliferated Kupffer cells in this condition. The splanchnic removal ratio, which can be determined by comparison of the bacterial concentration in hepatic vein to that in systemic blood, is an index of the function of the Kupffer cells and of the splenic reticuloendothelial cells. These cells do not remove all bacteria with equal efficiency [2232]. Passive and active immunizations increase the removal rate [1728]. To maintain a bacteremia in the peripheral blood, bacteria have to be continuously discharged, since the splanchnic reticuloendothelial system rapidly removes them. The gradual digestion of engulfed bacteria, protozoa (leishmania), or fungi (histoplasma) can be followed histologically.

BACTERIA IN LIVER. Horse, dog, and sheep livers normally contain anaerobic bacteria [2220]. These probably reach the organ by the portal circulation from the intestinal tract. The rat liver is free of bacteria [1319]. Bacteria, especially anaerobes, have been found after death in human [2233] and animal [2972] livers. Cultures of liver biopsies are generally negative [2805, 2898], but this may be the result of bactericidal action of fatty acids [2489]. Bacteria entering the portal circulation from the intestine under normal conditions, or as a result of intestinal irritation, are thought to be removed by the Kupffer cells. In dogs, however, as well as in man [2898], no bacteria are demonstrable even in the portal vein. Experimental hepatic damage leads to bacteria in the bile [2625] or lymph.

Phagocytosis of Blood Cells. Phagocytosis of erythrocytes and leukocytes by the Kupffer cells is not a normal pathway for their destruction. In arsine-intoxicated geese, engulfed red cells can be seen disintegrating within the Kupffer cells [2804]. A similar picture of erythrophagocytosis is found in man in sickle-cell anemia, in other hemolytic anemias, and in hemochromatosis [2797]. Phagocytosis of red cells following coating of single cells or clumps of cells has been visualized intravitaly [1809]. Coated cells are removed so rapidly from the blood that the sinusoidal blood flow is not impeded.

Leukocytes are engulfed, particularly in leukemia. Phagocytosis of eosinophils produces eosinophilic granules in the Kupffer cells.

Antibody Formation. Antibodies are probably formed in the reticuloendothelial system and in the plasma cells. Antibodies to azo dye proteins

are formed in the reticuloendothelial cells, specifically the Kupffer cells, and free globules may be visible around these cells during their formation [2862]. In this sense, the Kupffer cells represent clasmatocytes, which sacrifice some of their cytoplasm in antibody formation. Antigens and granular antigen-antibody complexes have been demonstrated by fluorescence methods in Kupffer cells [649]. Similarly, antigens have been localized by isotope methods [1003, 3491]. The specific sites within the Kupffer cells are probably the mitochondria [680]. Antigens have also been found in the mitochondria of the parenchymal cells [1003].

The relation of Kupffer cells and other hepatic mesenchymal cells to plasma cells, a well-established source of antibodies [895], is not known, especially when the cytoplasm of these cells in the liver is deeply basophilic. In septic conditions and in many liver diseases in man, and during antibody formation in rabbits, the cytoplasm of the mobilized and hypertrophic Kupffer cells is extremely basophilic, reflecting increased pentose nucleic acid, apparently as an indication of increased protein formation [896, 966, 1396, 3287]. Some of this protein may be antibody.

The nature of the antibodies formed by Kupffer cells is not well understood; some may be bacterial antibodies. Bacterial antibody production is increased in hepatitis [898] and cirrhosis [1429]. Specific antibodies have been described in hepatitis [290, 874], but their role is not established.

Globulin Formation. In liver disease, particularly cirrhosis and viral hepatitis, the serum-gamma globulin level is elevated [2636]. The gamma₁ globulin fraction, which is rich in antibodies, is especially high in cirrhosis [1073]. The hypergammaglobulinemia of cirrhosis has been reported by some to be associated with reduced turnover rates, which would indicate reduced destruction [1431], and by others with increased rates, reflecting increased formation [905].

Plasma cells in the bone marrow, spleen, and liver increase during gamma globulin formation after immunization [280, 287, 966]. Hypergammaglobulinemia is also associated with plasma cell hyperplasia in the bone marrow [2907], and agammaglobulinemia with absence of plasma cells.

Although plasma cells [288, 896] or lymphocytes [818, 1396] have been suggested as the site of gamma globulin formation, on the basis of

experiences with antibodies, reticuloendothelial cells with basophilic cytoplasm and without evidence of phagocytosis appear to be a more likely site [966]. Certain histologic alterations indicate that the liver is important in gamma globulin formation [236, 2907]. A fair correlation exists between the increase of cytoplasmic basophilia of the Kupffer cells and other hepatic reticuloendothelial cells and the increased serum-gamma globulin level. Moreover, radioisotope studies indicate that 80 per cent of the globulins under normal circumstances are formed in the liver and that therefore at least some of the gamma globulin must come from this organ [2295]. Perfusion studies, however, suggest that the liver normally makes little gamma globulin [2297]. Therefore, only circumstantial evidence exists that the Kupffer cells of the liver are engaged in gamma globulin formation. If they are, it is probably only under abnormal circumstances that this formation occurs.

The serum-gamma globulin elevation in liver disease is quantitatively much too high to be explained by increase of serum antibodies. The excess nonantibody gamma globulin, or reaction globulin [2221], seems to be an expression of irritation of the mesenchymal elements stimulated during antibody formation or by hepatic-cell breakdown products.

Metabolic Function. **FAT METABOLISM.** The Kupffer cells in man normally contain fat [1975], whereas little is found in dogs [2392] or guinea pigs [3640]. It is not related simply to the fat content of the hepatic cells. In hepatitis, the fat content of the Kupffer cells and hepatic cells is low [3433]. In cirrhosis, when the hepatic cells are rich in fat, Kupffer cell fat may be minimal. On the other hand in diabetes, infections, or cancer, the fat content of the Kupffer cells is high, although the hepatic cells may be free of fat [2797, 3433].

The role of the Kupffer cells in fat metabolism becomes apparent after injection of fat preparations. Fat preparations of previous years produced so much fat storage in the Kupffer cells that injection of fat emulsions was recommended as a test of reticuloendothelial function. The Kupffer cells took up the fat exclusively and supposedly transmitted it to the hepatic cells [1622]. Injection of dog chyle led to fat in the hepatic cells, in contrast to fat given intravenously which led to fat in the Kupffer cells [2392]. Better and newer preparations of fat for intravenous injection

bypass the Kupffer cells and reach the hepatic cells directly [3640]. Furthermore, blockade of reticuloendothelial cells by dyes prevents the deposition of fat in the Kupffer cells but does not alter the clearance of emulsified fat from the blood [3448]. These observations indicate that Kupffer cells have little part in the normal fat metabolism but are scavengers of unusable fat derived from exogenous sources or tissue breakdown.

Apparently, the role of the Kupffer cells in the metabolism of cholesterol and other lipids is similar. Colloidal cholesterol is engulfed by Kupffer cells but not by hepatic cells, indicating a scavenger activity rather than a normal metabolic pathway [267, 1921]. Lipids may be stored in the Kupffer cells in Gaucher's and Niemann-Pick disease and in Hodgkin's disease, producing the characteristic "foam" cells.

VITAMIN A. The fat droplets in the Kupffer cells, but not in the reticuloendothelial cells of the other organs, normally show considerable vitamin A fluorescence (Fig. 18), which is absent in nutritional deficiencies and in some forms of liver damage [2625]. In diabetes mellitus, uremia, and obstructive jaundice, the Kupffer cells show strong vitamin A fluorescence, whereas the hepatic cells are free of it. Whether this picture indicates impaired transmission to the hepatic cells or a scavenger function of the Kupffer cells after tissue breakdown is unknown. The vitamin A in the Kupffer cells may be in transit from the blood stream to the liver or, as with fat, may be unusable material which has been engulfed. In rats on vitamin A-deficient diets the fluorescence disappears last from the Kupffer cells. In depleted rats, the fluorescence after administration of vitamin A appears first in the Kupffer cells, suggesting a distributing role of the Kupffer cells in vitamin A metabolism [2625]. Hepatic vitamin A stores are reduced after reticuloendothelial blockade, although this has been denied [3366]. In contrast, if large amounts of vitamin A are given to rats, vitamin A fluorescence in the hepatic cells increases only slightly, whereas the Kupffer cells become exceedingly rich in vitamin A [2628] (Fig. 34, lower right). Under these circumstances, the rat liver stores enough vitamin A to maintain the animal for a century. Nevertheless, on a subsequent vitamin A-deficient diet it is quickly lost [920], a finding which has been interpreted as destruction of vitamin A by the Kupffer cells. Since only vitamin A alcohol which has passed through the hepatic cells is utilized by the body,

excess vitamin A ester ingested is stored in the Kupffer cells [1192], which do not contain lipase and therefore do not split it to form the free alcohol. Under normal circumstances the Kupffer cell vitamin A therefore represents an unusable excess, comparable to Kupffer cell fat. In cirrhosis, histiocytes in the portal tracts contain vitamin A [2625], probably owing to phagocytosis of fat and vitamin A-containing tissue fragments.

BLOOD PIGMENT BREAKDOWN. The role of the Kupffer cells in blood pigment breakdown and bile pigment metabolism (see Bilirubin, Chap. 11) is fourfold: (1) they engulf abnormal red cells; (2) they participate with other parts of the reticuloendothelial system in the formation of bilirubin; (3) they possibly change the physicochemical characteristics of bilirubin to cause a direct van den Bergh reaction; (4) they store iron.

Excessive destruction of blood, faulty utilization of iron in hematopoiesis, and intravenous administration of blood or colloidal or saccharated iron [943] results in iron storage in the Kupffer cells. Only subsequently is some iron stored in hepatic cells (hemosiderosis; Fig. 34, upper right; see Iron-storage Diseases, Chap. 53). In disturbances of cellular iron metabolism, as in hemochromatosis, iron appears first in the hepatic cells and then in the Kupffer cells. In the early stages of malnutrition, iron pigment appears first in the hepatic cells [1172]. Later it appears in the Kupffer cells and in the cells of the portal tract, at the same time decreasing in the hepatic cells. These findings possibly are the result of breakdown of hepatic cells and phagocytosis of breakdown products containing iron in enzymes, such as the cytochromes, or of excessive intestinal reabsorption of iron excreted into the bile [1172]. The presence of iron in the Kupffer cells is probably not an expression of their metabolic function but rather of simple phagocytosis.

OTHER METABOLIC FUNCTIONS. The Kupffer cells have been implicated in the metabolism of other substances, such as carbohydrates, proteins, water, and minerals. Definite proof of this role is lacking.

Proliferation and Regeneration. The reticuloendothelial system has a greater proliferative capacity than almost any other tissue in the body. The intralobular typhoid nodule is an example of the rapid proliferation of hepatic reticuloendothelial cells. The proliferations become very large and even form gross nodules in reticulum cell sarcoma, Hodgkin's disease; and non-lipid-storing

reticuloendotheliosis of the Letterer-Siwe type. These cells may represent the source of reticuloendothelial cells, such as the monocyte, in the blood stream.

Kupffer cells which contain small amounts of injected colloids or india ink may persist for a long time. If, however, large amounts of these materials, particularly colloidal metals, are injected intravenously, Kupffer cells which have engulfed the material are rapidly discharged, and a new generation quickly develops. A similar proliferation followed by discharge and regeneration occurs with any type of damage or inflammation and has been produced by injection of foreign proteins or diphtheria toxins [436].

Blockade or Exhaustion. The claim has been made repeatedly that phagocytosis of large amounts of dyes or other material renders the reticuloendothelial cells incapable of accepting additional material. This blockade is usually incomplete, although colloidal copper may produce a complete blockade for a few hours at least. However, vital microscopy does not provide evidence that the Kupffer cells become unable to accept additional material which is properly coated. Moreover, whether the blockade represents an exhaustion of Kupffer cells or an excessive discharge of Kupffer cells without replacement is not clear. Apparent blockade following the injection of india ink may be the result of a toxic factor in commercially available preparations, rather than of mere storage of the carbon particles [3716].

PHAGOCYTOSIS. Removal of pyrogens from the circulating blood is retarded by reticuloendothelial blockade [203], but in bacteremia an exhaustion of the mechanism for splanchnic removal of bacteria is not demonstrable [2232].

ANTIBODY FORMATION. After partial blockade, mild infections may become serious [2565]; for example, bartonella infection may be fatal, and resistance to streptococci is decreased [1172]. Anaphylactic or histamine shock is lessened or prevented by blockade of the reticuloendothelial system, and the Schwartzmann phenomenon is depressed [203].

BILE PIGMENT METABOLISM. Blockade of the reticuloendothelial system is said to inhibit bile pigment formation [943]. "White bile" after blockade, however, is possibly the result of associated hepatic dysfunction.

METABOLIC FUNCTION. Extensive alterations of metabolic functions have been reported, but few

reports have been substantiated [1172]. Protein, fat, and carbohydrate metabolism are supposedly affected, and hypercholesterolemia is said to appear. The inactivation of gonadotropic and thyrotropic hormones is retarded during blockade [1228]. After "irritation" of the reticuloendothelial system by injection of trypan blue, animals are more sensitive to estrogens, and their adrenal glands become hyperactive [1172].

Tests of the Reticuloendothelial System. The clearance of congo red from the serum has been described as an indication of the function of the reticuloendothelial system [3207]. Since congo red is bound to albumin, objections to the test have been raised [1962], and today it is rarely used for this purpose. Recently, injection of chromium phosphate tagged with radioactive phosphorus has been used for similar purposes [2707].

The biliary tree is a channel which delivers bile from the hepatic cells to the duodenum and which has a side arm, the gallbladder. With the exception of this side arm, the biliary tree has no influence upon the constitution of the bile other than the addition of some mucus.

The biliary system, beginning at the bile canaliculi between the hepatic cells and ending at the papilla of Vater, is composed of a series of tubes, which progressively increase in caliber.

Bile Canaliculi

Bile canaliculi, or capillaries, are fine tubes between neighboring hepatic cells (Fig. 36, upper left). Their walls are thin, and many deny their existence [2126], although they have been separated after maceration of hepatic tissue [1130] or by teasing [907]. Whether the bile canaliculus wall should be considered a specially adapted, detachable part of the hepatic cells or an independent entity is not established. Fine structural details, such as an acidophilic lining or a brush border, have been described [2358]. The wall of the canaliculus can be demonstrated by such stains as the phosphotungstic acid hematoxylin method, Mallory connective tissue stains, and by silver impregnation [1903, 2188]. Although the presence of a wall apart from the hepatic cell seems probable, it is of questionable significance, because when hepatic cells disappear, so do the bile canaliculi. They lose their staining properties if the hepatic cells are damaged. Intracellular secretory ramifications or diverticula of the canaliculi have been described, primarily on the basis of Golgi preparations [2126]. With other methods, however, such diverticula can not be seen, and in injection preparations, the outlines of the bile canaliculi are straight [907]. Diverticula in di-

lated canaliculi after experimental biliary obstruction have been visualized by alkaline phosphatase stains [3447].

In injection as well as tease preparations, the bile canaliculi form a polygonal network with many intercommunications (Fig. 35*B, C*).

Originally this network was considered the core, or axial canaliculus, of the hepatic-cell cords, separated from the blood capillary network by one hepatic cell. The new concept of hepatic-cell plates describes a network of bile canaliculi within the plate, one hepatic cell filling each mesh [907, 1379] (Fig. 35). The hepatic cells, depending on their shape and position in the plate, have differently shaped grooves on their surfaces to contain these bile canaliculi. Only occasionally is a bile canaliculus on the surface of the plate next to the sinusoid [884], after disappearance of an intervening hepatic cell. After injection of fluorescent dyes, the bile canaliculi during vital microscopy appear to be a continuous system with local widenings [1066, 1241]. Intracapillary vacuole formation, or extensive sprouting [1502], is probably abnormal.

Bile Ductules

The bile canaliculi drain into the smallest bile ducts, the anatomic structure and even nomenclature of which are not agreed upon (Fig. 36, upper right). The initial narrow portion of the bile ducts, for which the terms "cholangioles," "septal bile ducts" [2083], "*Zwischenstücke*" (intermediary portions) [586], and "terminal ductules" [907] have been proposed, can best be designated simply as bile ductules, analogous to arterioles. The junction of ductules and bile canaliculi may be dilated; this portion has been called the ampulla [943] or canal of Hering [2249].

However, whether the dilatation involves the initial portion of the ductule or the terminal portion of the bile canaliculus is not known, especially where several canaliculi merge before entering the ductule [2126]. The term "canal of Hering" is often applied to the entire ductule.

The ductules have a cuboidal to low columnar

epithelium, with chromatin-rich nuclei and light chiefly eosinophilic cytoplasm [586]. Mitoses are rare, and, in contrast to the hepatic cells, the cuticular border can not be stained by bile canaliculus stains and a basement membrane is demonstrable by silver impregnation or Mallory's aniline blue stains. Ductular epithelium differs

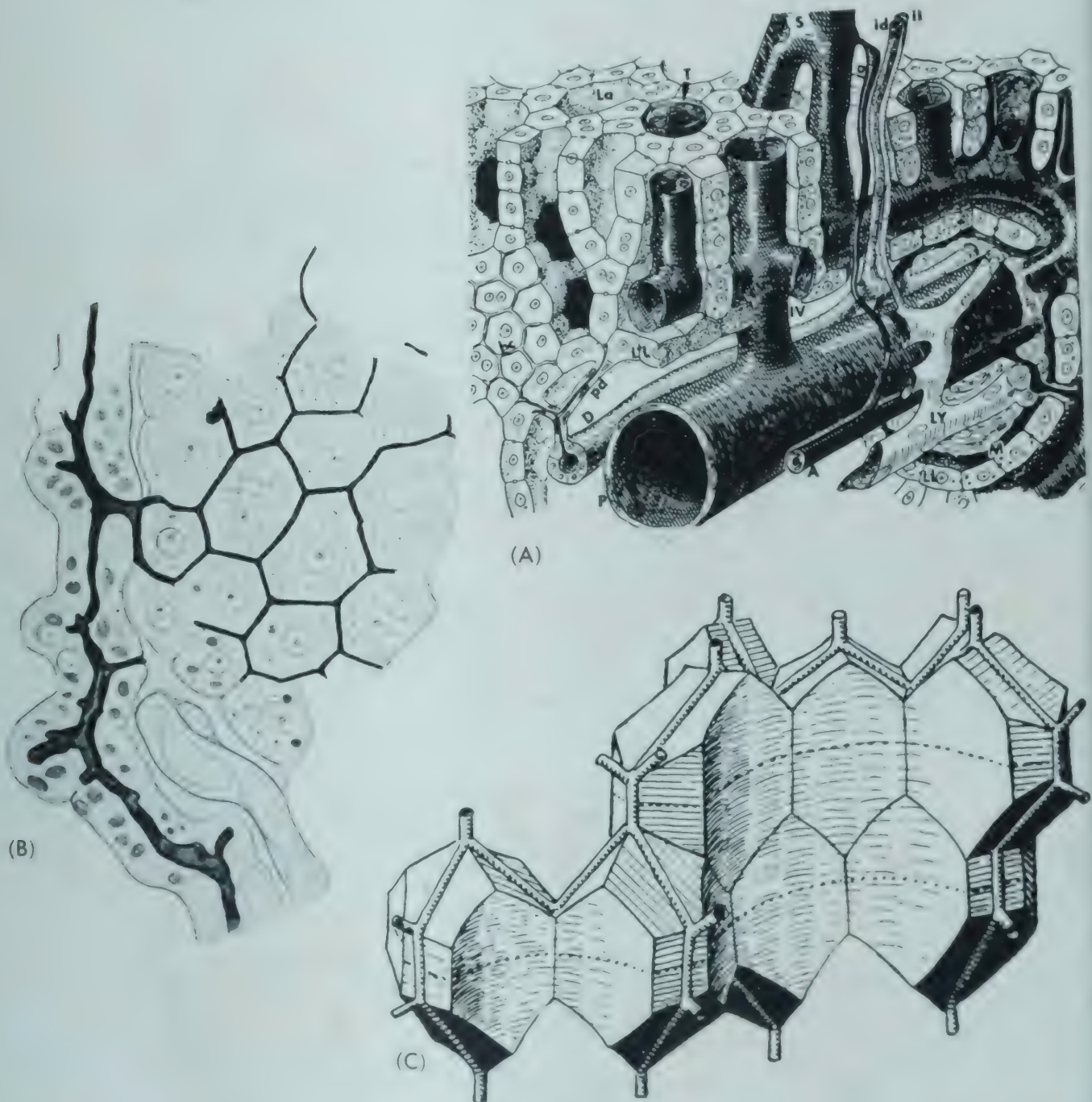


FIG. 35 (A) Stereogram of the normal liver. A, hepatic artery branch; bc, bile canaliculus; D, interlobular bile duct; ia, intralobular arteriole; id, intralobular ductule (cholangiole); il, intralobular lymph vessel; IV, inlet venule; K, Kupffer cell; La, labyrinth; LL, limiting plate; LY, portal lymph vessel; P, portal vein branch; pd, periportal ductule; S, sinusoid; T, tissue space of Disse. (Popper, H.: *Am.J.Med.* 16:98, 1954.) (B) Bile canaliculi of a dog in a hepatic plate draining into an intralobular ductule. (From original of Fig. 47 Elias, H.: *Am.J.Anat.* 85:447, 1949.) (C) Arrangement of the hepatic cells and bile canaliculi in a hepatic cell plate. (From original of Fig. 60, Elias, H.: *Am.J.Anat.* 85:453, 1949.)

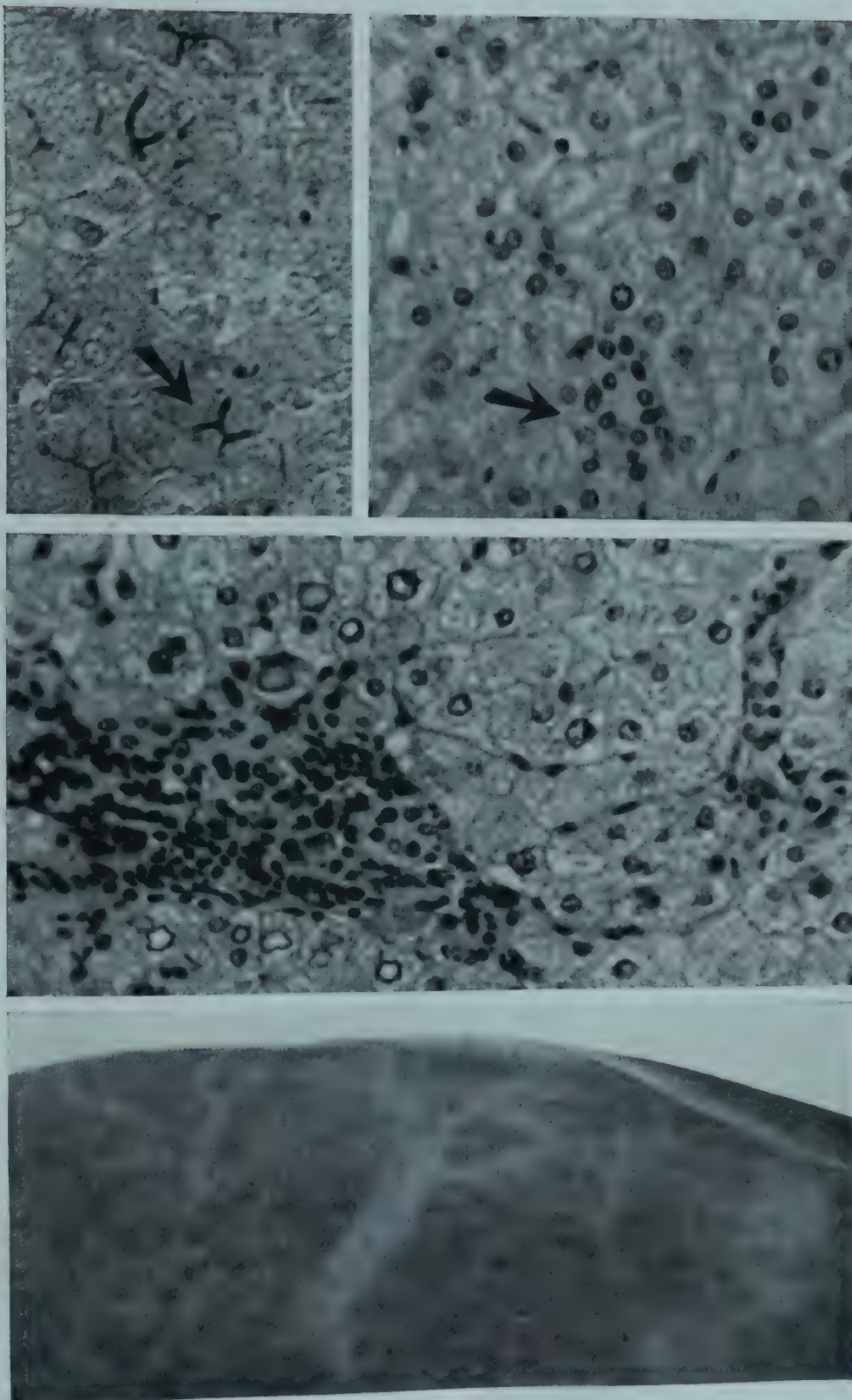


FIG. 36 *Upper left.* Dilated bile canaliculi in hemolytic jaundice. H&E ($\times 230$). *Upper right.* Intralobular ductule. H&E ($\times 330$). *Center.* Intralobular and peri-lobular ductules, the latter surrounded by inflammatory exudate in a small portal tract. H&E ($\times 330$). *Bottom.* Close-up of capsule of liver in extrahepatic biliary obstruction showing dilated subcapsular vestigial ducts.

from hepatic cells by having no mitochondria, and from that of interlobular ducts by taking Eppinger's bile canaliculus stain but failing to show a mucoid lining stainable with PAS [2263]. The distensibility of the ductules, with the possibility of rupture under abnormal circumstances, explains Aschoff's term "heel of Achilles." The ductules connect either with the bile canaliculi in the limiting plate or with bile canaliculi in the hepatic plates within the lobule, either at slight angles, as indicated by many published illustrations [586, 2249], or perpendicularly [907] (Fig. 35A and B). The length varies; those which connect the limiting plate canaliculi with the interlobular bile ducts in the portal tract (perilobular ductules) are very short, while those which extend to the central portion of the lobule (intralobular ductules) are relatively long and form loops communicating with one another (Fig. 36, middle). The intralobular ductules are usually accompanied by an arteriole and are surrounded by a delicate connective tissue mantle in at least part of their length (Fig. 115A). Whether more bile drains through the short ductules in the limiting plate or through the longer intralobular ductules is not known. The junction of the ductules and the smallest bile ducts is not clearly defined, and the structures called the perilobular bile ducts [2156] may actually represent ductules.

Intrahepatic Bile Ducts

The interlobular bile ducts connected to the bile ductules are in the smallest portal tracts and anastomose freely (Fig. 60). Each duct drains a given segment of the liver, and accessory ducts are aberrant segmental ducts [1437]. The ducts have a wider lumen than the ductules and are lined by a uniform layer of low columnar epithelial cells, with lightly staining cytoplasm and nuclei close to the basement membrane. The cytoplasm is normally free of structural details and basophilic granules [3287]. Under abnormal conditions in man, it contains fat droplets and iron. In some species, such as the dog, fat is normally present. A basement membrane demonstrable by silver impregnation or connective tissue stains merges with a well-defined layer of collagenous fibers, which is a circular reinforcement of the fibers present in the portal tract.

The walls of large bile ducts in larger portal tracts have a high cylindrical epithelium with a thin basophilic layer composed of mitochondria below the nucleus. A similar thin layer just be-

low the surface has been mistaken for a cuticular membrane. Cholesterol crystals may be found. Lymphocytes are often seen between the epithelial cells. The relatively thick wall contains elastic fibers.

The larger intrahepatic bile ducts have outpouchings lined by mucus-producing epithelium, sacculae, and are surrounded by vascular plexuses. The bile ducts eventually unite to form area ducts of the third order, which in turn unite to form segmental ducts of the second order. These join and form the lobar ducts of the first order, the right and left main branches of the common hepatic duct [1437] (Fig. 64). The caudate lobe is drained by ducts from the right and left lobar ducts. This does not produce effective communication between the right and left lobes. Except for small extrahepatic or capsular anastomoses, which occur in one-fourth of persons, no other communications exist [1437].

VARIATIONS. The ramifications of the draining bile ducts vary greatly, especially in the right lobe. Aberrant ducts may connect with extrahepatic bile ducts and are usually not accessory ducts but rather the draining duct of the respective area.

Vestigial Bile Ducts. Ramified bile ducts are found immediately below the capsule of the liver. These frequently connect with deeper bile ducts. They represent rudimentary or vestigial ducts around which the lobular parenchyma never developed or has disappeared. In the fibrous appendix on the lateral edge of the left lobe such bile duct remnants may form an extensive network. In obstructive jaundice the vestigial bile ducts become conspicuous because of dilatation and may rupture or be punctured during liver biopsy (see Dangers, under Indications, Contra-indications, and Dangers, Chap. 39). Spontaneous or traumatic rupture causes biliary peritonitis (Fig. 36, bottom).

Extrahepatic Bile Ducts

GROSS ANATOMY. At the porta hepatis, the ducts from the right and left lobes fuse to form the main hepatic duct, which is approximately an inch or less in length (Fig. 37). After junction with the cystic duct it becomes the common bile duct, which varies in length because of variations of the cystic duct. The over-all length of the extrahepatic bile ducts is about 4 to 5 in., and the average diameter is about $\frac{1}{4}$ in.

From the porta hepatis, the extrahepatic bile

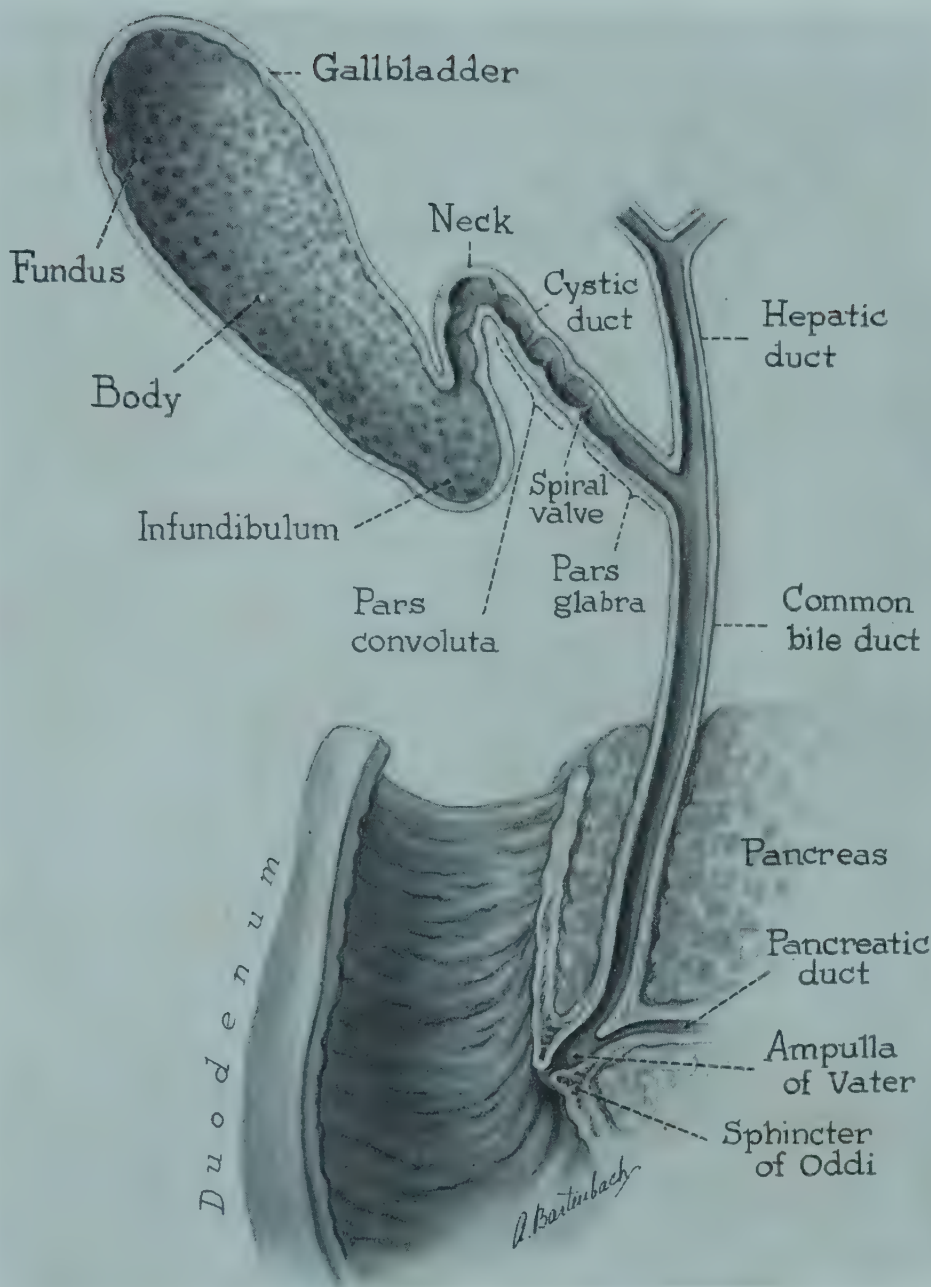


FIG. 37 Drawing of gallbladder and extrahepatic biliary tract.

ducts descend along the free margin of the lesser omentum to the upper edge of the first portion of the duodenum. The common duct crosses the duodenum from behind and is located either in a groove or in a tunnel in the upper posterior part of the head of the pancreas. This portion of the duct is relatively thin walled and wide and has a smooth mucosa. The duct then passes obliquely through the wall of the second portion of the duodenum and ends in a mucosal elevation, known as the papilla of Vater (Fig. 37). The common duct sometimes narrows before its entrance into the papilla, which may result in impaction of stones at this point. Four portions of the duct are thus recognized: supraduodenal, retroduo-

denal, infraduodenal or pancreatic, and intra-duodenal. Variations of the extrahepatic bile ducts are common and are discussed together with those of the cystic duct (see Cystic Duct, further on in this chapter).

MICROSCOPIC ANATOMY. The surface epithelium of the extrahepatic ducts is up to $50\ \mu$ in height and contains cholesterol, indicating absorption. The ducts themselves do not secrete anything, but glandlike appendages secrete mucin. These glands are tortuous, ramified tubes, separated from each other by connective tissue layers, and they extend through all layers of the wall. In the mucosa, lymphocytes and occasionally segmented leukocytes are found. The mucosa is thrown into

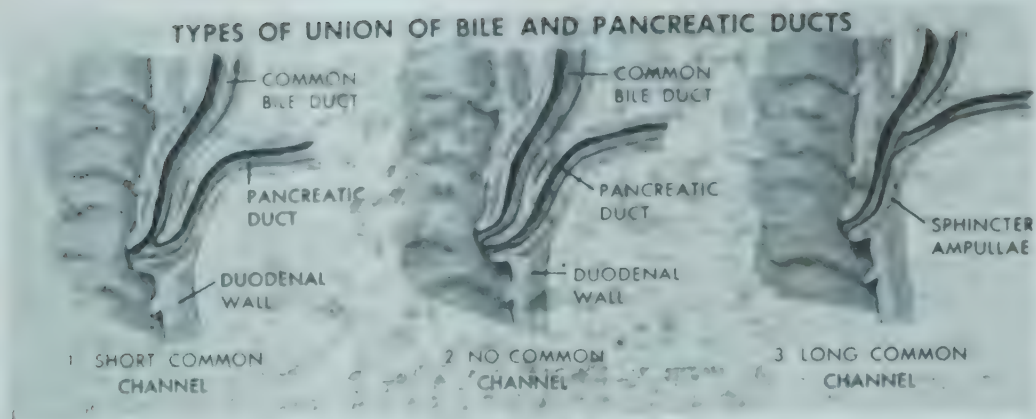


FIG. 38 Types of union of bile duct and the main pancreatic duct at the ampulla of Vater. (Copyright © The Ciba Collection of Medical Illustrations, Frank H. Netter, M.D.)

many folds so that the lumen of the duct is star-shaped on cross section. The wall is reinforced by interlaced collagenous fibers mixed with a dense network of elastic fibers. A few smooth-muscle bundles contribute little to this reinforcement. They have longitudinal and oblique arrangements and form a continuous layer only near the entrance into the duodenum. In two-thirds of hepatic ducts examined and in 7 per cent of common ducts examined, muscle bundles were absent.

Termination of the Common Duct

AMPULLA. A dilatation in the terminal portion of the common duct is called the "ampulla," although some use this term when referring to a common termination of the pancreatic and common bile ducts. This confusion in terminology led to divergent anatomic statistics; the existence of a true ampulla has even been denied [1773, 3201]. One investigator found it in 69 per cent of cases examined, although it measured less than 2 mm in length in 45 per cent of the total number, and 5 to 10 mm in length in only 3.5 per cent [2203]. Other investigators recognized the presence of an ampulla in only 5 to 8 per cent of cases examined [724].

VARIATIONS OF AMPULLA. The terminal portion of the common duct in the duodenal wall is funnel-shaped and curved, and it frequently interweaves with the pancreatic duct [3201]. The junction of the common and pancreatic ducts with the duodenum follows one of three patterns (Fig. 38):

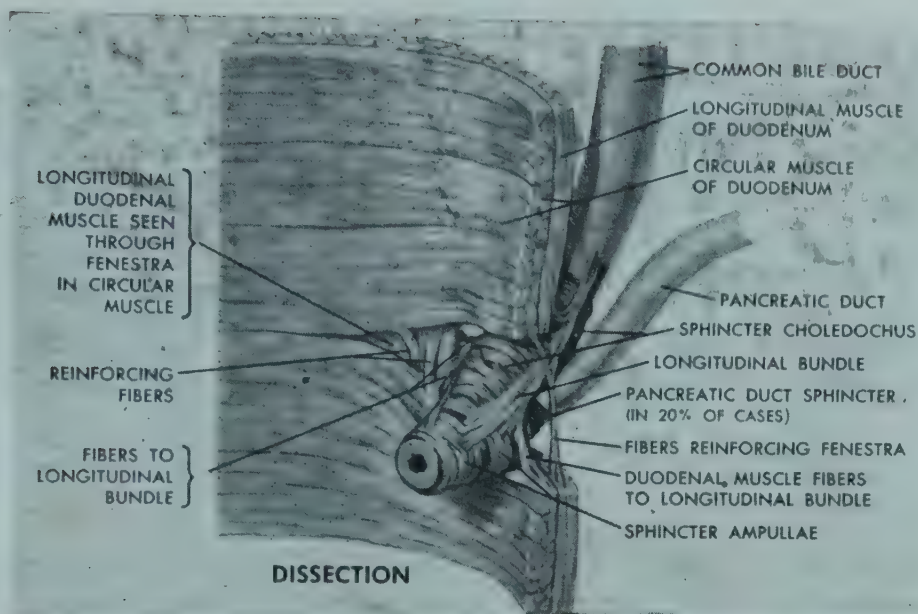
1. The ducts unite outside the duodenum and traverse the duodenal wall and the papilla as a single duct.

2. The ducts join within the duodenal wall or papilla and have a short common terminal portion [2474].

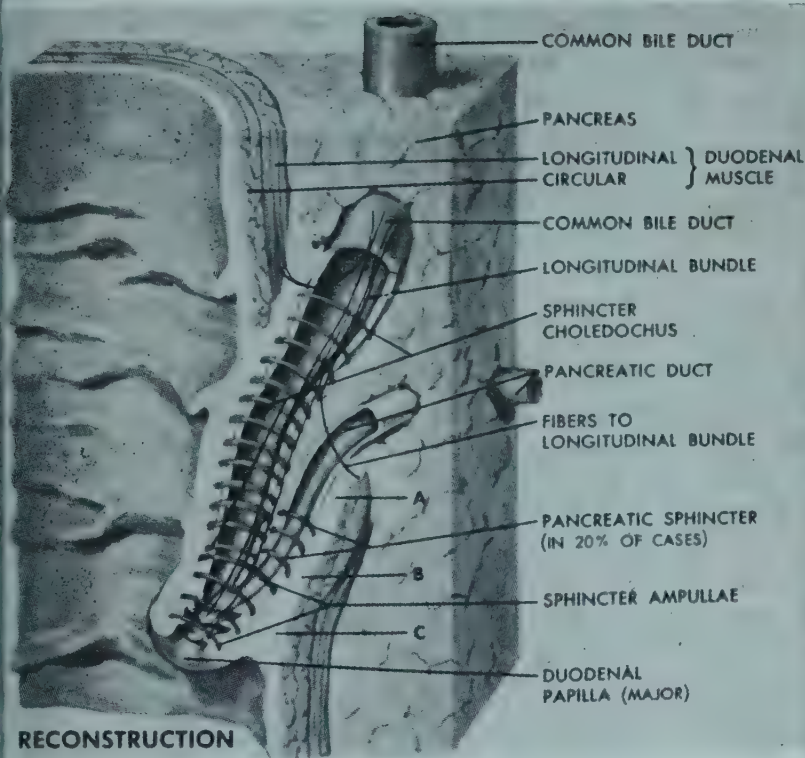
3. The ducts have independent openings at the papilla of Vater, or, occasionally, at some distance from one another.

The crucial question from a functional point of view is whether the opening permits a communication between the pancreatic and common bile ducts and thus permits reflux from the pancreas into the biliary tract or vice versa. Such a "common channel" is of major significance if the opening of the papilla is obstructed by a stone or muscular spasm [283]. Whether such a channel is considered functioning depends upon evaluation of the anatomic findings and mode of examination in the group with a short common terminal portion. Two recent reviews based upon almost the same literature come to divergent conclusions [1556, 3201]. One reviewer claimed that in 1,252 cases collected from the literature, separate orifices were found in 29 per cent, whereas 64 per cent was found in his own material. With injection techniques on fresh specimens the possibility of a common channel was found in at least half the cases [1556]. Variations in the length of the common portion in fixed specimens assumed to be necessary for a common channel are responsible for some differences in reported statistics. If the total length of the papilla is more than twice the length of the common portion, a closed common channel can not be formed by sphincter action, and if this group is excluded, reflux is possible in 20 per cent of individuals [3201, 3202]. Radiographically, reflux from the common bile duct into the pancreatic duct was noted in about 16 per cent of cases examined [1556, 3201].

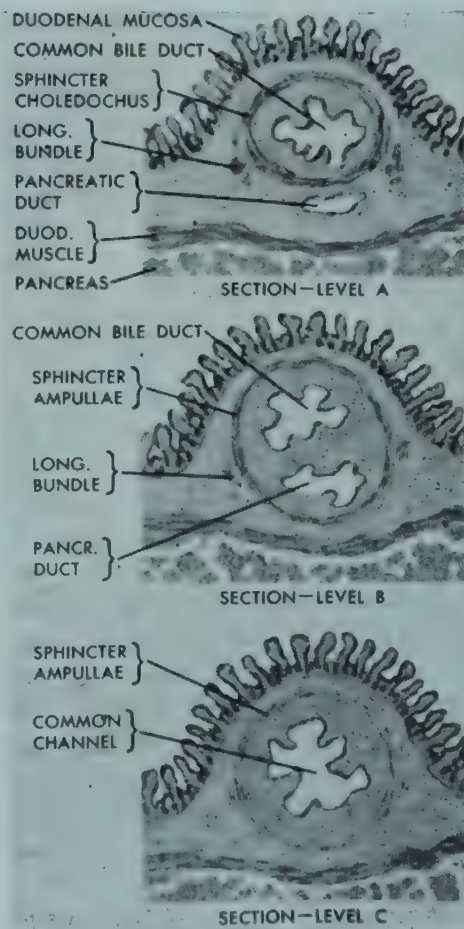
SPHINCTER OF ODDI. The sphincter system surrounding the common and pancreatic ducts at the papilla of Vater is the sphincter of Oddi. The ducts surrounded by the sphincter pass through



A



B



C

FIG. 39 Drawing of sphincter of Oddi. (Copyright © The Ciba Collection of Medical Illustrations, Frank H. Netter, M.D.)

an eye-shaped slit in the duodenal musculature, the fenestra choledocha [365]. The split is lengthwise in the longitudinal layer. The size of the fenestra determines the degree of interference with bile flow due to duodenal tonus and peristalsis and the size of calculi which can enter the

duodenal wall. In contrast to current opinion, some have claimed that the sphincter system is only part of the duodenal musculature. The intrinsic muscles of the ducts and ampulla are the following [1865] (Fig. 39):

1. Longitudinal fasciculae extend between both

ducts from the fenestra to the ampulla, connecting the two ducts with each other as well as with the duodenal muscles coming from the aperture. They shorten or erect the papilla, thus permitting a rapid flow of bile.

2. The sphincter choledochus is an annular sheath which surrounds the common duct from outside the fenestra choledocha to the junction with the pancreatic duct. It controls the flow of bile and when contracted permits filling of the gallbladder.

3. The sphincter pancreaticus, present in only one-third of all adults, surrounds the preampullary portion of the pancreatic duct and controls the flow of pancreatic juice.

4. The sphincter ampullae, present in only one-third of all adults, consists of a relatively weak annular sheath beginning just before the junction of the ducts and continuing almost to the end of the ampulla when the latter is present. Spasm of this muscle may produce a common channel with reflux of pancreatic juice into the biliary tract or of bile into the pancreatic duct system.

In addition, fibers derived from the duodenal muscles reinforce angles of the duodenal window, preventing its enlargement, and connect with the duodenal muscles for the purpose of erecting the papilla.

As a whole, the sphincter of Oddi, surrounding mainly the submucosal part of the duct, is not only able to occlude the pancreatic and common bile ducts but may also assist in the discharge of fluid from the duct by the longitudinal fasciculae erecting the papilla.

Gallbladder

The gallbladder represents a side arm to the biliary system connected to the common duct by the cystic duct. Some animals, such as the whale, elephant, hog, camel, male giraffe, deer, horse, and rat, have no gallbladder and also no sphincter of Oddi.

GROSS ANATOMY. The bladder itself is pear-shaped. It consists of four parts (Fig. 37). The plump, blind end, usually flush with the liver edge, is called the "fundus." The kinked part of the fundus is called a "phrygian cap." The main portion, called the body, is followed by the infundibulum, or Hartmann's pouch, which runs parallel with the cystic duct and is bound to the duodenum by the cholecystoduodenal ligament. Finally, the neck, the pointed portion directed

toward the porta hepatis, leads into the cystic duct. The gallbladder is located in the grooved gallbladder bed of the liver, which consists of a loose connective tissue containing lymphatic and blood vessels. The gallbladder varies in size and shape; its capacity is 1 to 2 ml per kilogram of body weight [2249]. The mucosa is usually thrown into folds, which are subdivided into irregularly arranged smaller folds, depending on the stage of contraction. The folds may disappear with overdistention of the gallbladder.

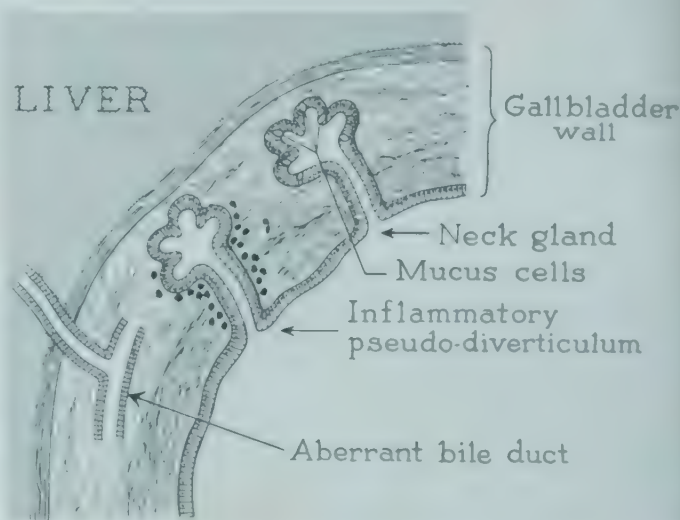
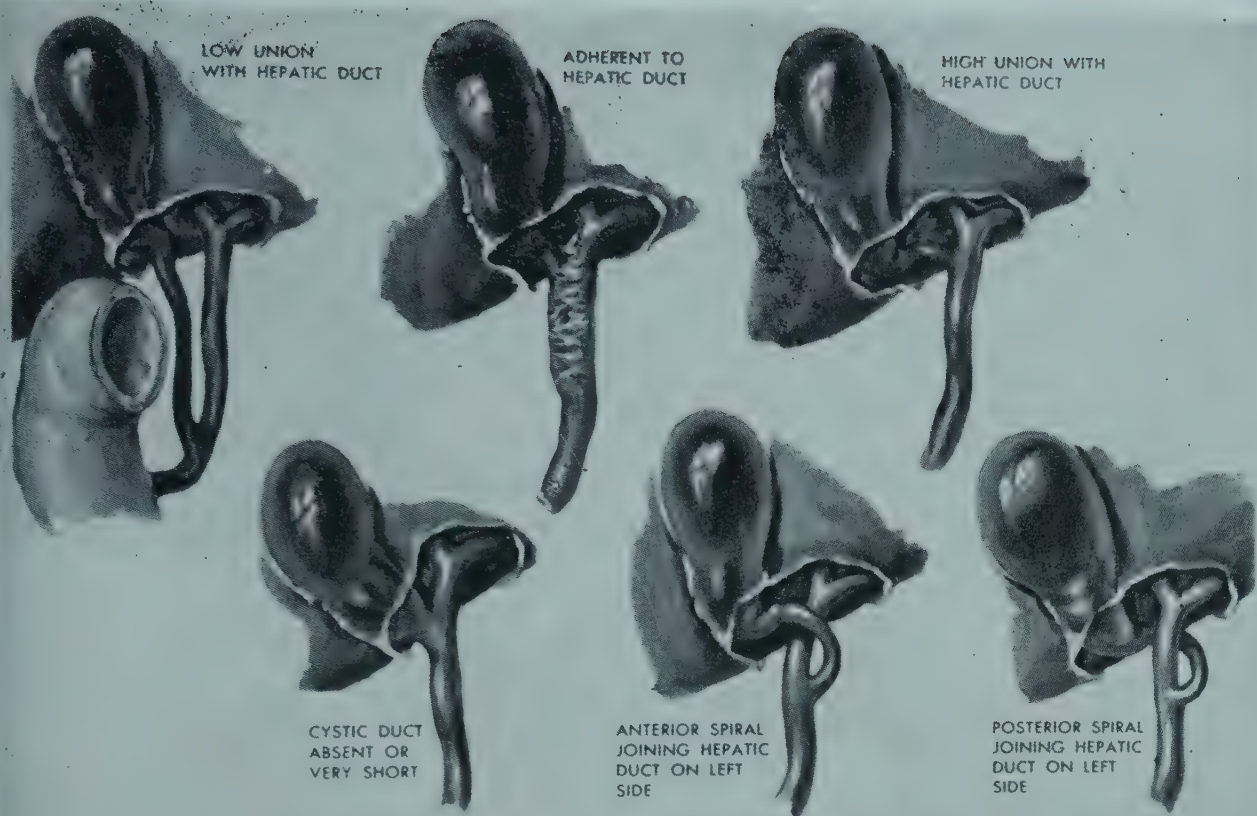


FIG. 40 Ductal and glandular structures found in the gallbladder wall.

MICROSCOPIC ANATOMY. A high columnar epithelium with oval, chromatin-poor nuclei lines the wall. The cytoplasm is slightly eosinophilic and may contain neutral fat and other lipids, especially cholesterol. The mitochondria are distributed below the surface as well as in the base of the cells. A cuticular layer consists of surface filaments [2704]. Other details are considered variations during the secretory process [997]. Goblet cells as well as glands are absent, except in the neck. Instead of having a well-defined submucosa, the epithelium is based upon a loose lamina propria which is moderately vascular but has few cells. This layer in turn is based upon an irregularly arranged muscular layer, which is sparse in man and contains many elastic fibers and collagenous bundles. The layers are not well separated, and the internal muscle bundles, chiefly longitudinal, are derived from an external bundle meshwork which is interwoven with a collagen and elastic network. The outer layer is composed of dense collagenous connective tissue with few elastic fibers, and merges with the



ACCESSORY HEPATIC DUCTS

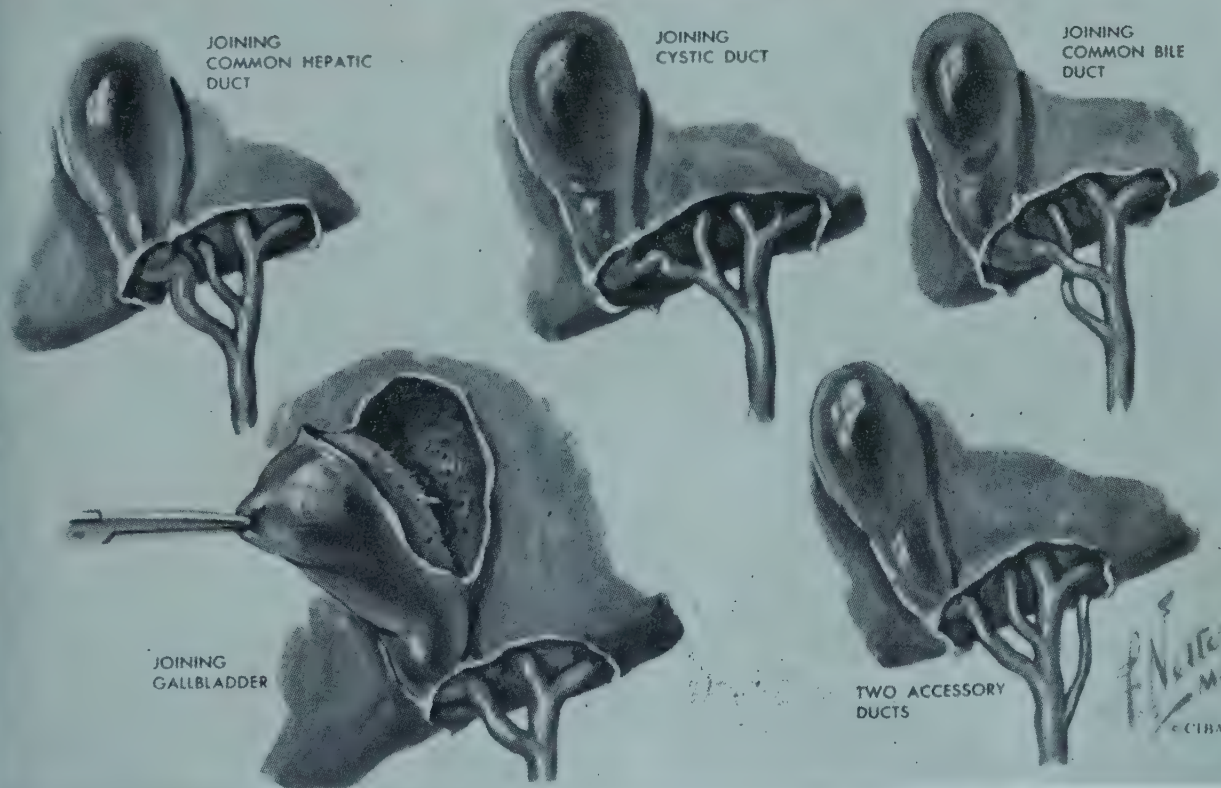


FIG. 41 Variations of the cystic duct and accessory hepatic ducts. (Copyright © The Ciba Collection of Medical Illustrations, Frank H. Netter, M.D.)

gallbladder bed or is covered by peritoneal endothelium. It contains fat cells, lymphoid and histiocytic elements, and the supporting vascular and neural structures.

The derivation and significance of epithelial structures in the wall of the gallbladder in pathologic processes have caused much controversy, resulting in confusion in nomenclature, especially in the use of eponyms such as Rokitansky, Luschka, and Aschoff. On the basis of thorough investigations [1360, 2785], these structures are divided into three groups.

1. Near the neck, in the lamina propria and the muscular layers, a few simple tubular alveolar, mucus-producing glands are found which have basophilic and oxyphilic granules [1360]. Their epithelium is cuboidal, and their nuclei are dark and near the base of the cell, easily differentiating them from the surface epithelium.

2. In addition, in all parts of the gallbladder, glandlike slits are noted as outpouchings of the surface epithelium which may extend through all the layers of the gallbladder wall into the fibrous or subserous layers. These diverticula dilate, particularly as the result of increased intracystic pressure, and may contain inspissated calculous material which irritates the epithelial lining and leads to proliferation with formation of adenomalike structures. The original communications with the lumen may become obliterated, and cyst formation occurs. These diverticula are found in 50 per cent of gallbladders after thirty years of age and are probably caused by factors such as increased cystic pressure, lack of muscularis mucosa, irregularity of the arrangement of the muscle fibers, and isolated responses of the muscle bundles to stimuli [2785].

3. Particularly in the gallbladder bed, aberrant bile ducts similar to those found in the capsule of the liver are found, occasionally surrounded by hepatic tissue. They may secrete bile into the wound after cholecystectomy.

The eponyms can be replaced by the following three terms: (1) neck glands; (2) inflammatory pseudodiverticula; (3) aberrant bile ducts (Fig. 40).

Cystic Duct. The tortuous cystic duct is about 1 in. in length and is histologically similar to the

rest of the extrahepatic bile ducts. The tortuosity is produced by mucosal folds containing spirally arranged smooth muscles, which produce the heisterian valves (Fig. 42D). The arrangement of the valves is such that the gallbladder fills under low pressure but empties only under high pressure. The junction between the cystic and common ducts shows great variation [727]. Three main types are found: angular; parallel, where the two ducts run side by side for a short distance; and spiral, where the cystic duct winds itself around the common duct.

VARIATIONS. The variations of the cystic duct are surgically important, since ligation of an unrecognized duct may be the cause of postoperative leakage. It may also be responsible for division or injury of the common or hepatic ducts, with resulting stricture formation [1437, 1994, 2284] (Fig. 41). The site of the union between the cystic and hepatic ducts may be very low near the duodenum, in which case the supraduodenal portion of the common duct is almost completely missing and the cystic and common hepatic ducts have long parallel courses. Sometimes the ducts are held together by a common connective tissue sheath; a stone in the cystic duct may thus compress the common duct. The cystic duct may join the hepatic duct close to the liver and may be so short that the gallbladder seems to enter directly into the hepatic duct. While the cystic duct usually joins the right aspect of the hepatic duct, exceptionally it spirals anteriorly or posteriorly around the hepatic duct to enter its left aspect. Accessory hepatic ducts drain circumscribed portions of the liver and therefore do not functionally duplicate other channels. They are found more frequently on the right side and are readily confused with the cystic duct, especially if it crosses the cystic triangle of Calot. Most commonly an accessory duct enters the common hepatic duct and less often the cystic or common bile duct. If it enters the common bile duct it crosses the cystic duct. An accessory duct may also run through the gallbladder bed and even enter the gallbladder lumen. If such a duct is not ligated during cholecystectomy, bile may leak into the gallbladder bed postoperatively and cause abscess formation or peritonitis.

The function of the biliary system has been studied extensively, primarily from the point of view of the gallbladder and its disease [1603, 2202, 3477]. Here the processes which directly concern hepatic function and structure are emphasized.

Intrahepatic Bile Ducts and Biliary Pressure

The intrahepatic ducts act as a channel system that normally has little effect on the constitution of the bile. The few adnexal sacculae dilate after cholecystectomy or biliary obstruction, suggesting the assumption of some storage function. Moreover, the presence of mucus glands suggests admixture of small amounts of mucus. The total volume of fluid contributed by the entire biliary conducting and storage system does not exceed 20 ml; the contribution of the intrahepatic portion is probably insignificant.

The biliary pressure in the intrahepatic and extrahepatic system up to the papilla of Vater, exclusive of the cystic duct and gallbladder, is the result of several forces. The secretory pressure of the liver amounts to approximately 30 cm bile in dogs and man [1605] and 23 to 36 cm in rats [1101]. The pressure is higher during inspiration than in expiration and fluctuates greatly during coughing, sneezing, or laughing. The sphincter of Oddi offers a resistance of 9 to 23 cm water, usually 12 to 15 cm, as measured by placing a T-tube in the common bile duct [228, 1605] or in the presence of traumatic biliary fistulas [2861]. The effect of gallbladder function is discussed later. Elevation of the biliary pressure above 35 cm owing to obstruction or spasm suppresses biliary secretion and results in the characteristic clinical symptoms of pain, nausea, vomiting, and limitation of motion of the right diaphragm

[2888], especially when the pressures fluctuate widely, as they do in inflammatory conditions. Pain can be abolished by splanchnic section, while vagotomy abolishes the nausea and vomiting; abolition of the associated respiratory disturbances requires section of both splanchnic and vagus nerves. Prolonged but lesser biliary pressure causes anorexia as a result of inhibited contraction of the stomach [1605]. In cholangitis, pain is elicited at pressures as low as 7.0 cm water. With disappearance of the infection after continued drainage of the ducts and antibiotic therapy, the pressure at which pain is produced increases [2888].

Gallbladder

The gallbladder has several functions relative to the flow and the constitution of bile.

Storage of Bile. Bile is produced by the liver at a constant rate in amounts almost approximating that of urine production. Since the bile serves not only as an excretion but as a secretion, a constant flow of bile into the duodenum would not be efficient for the absorption of fat or fat-soluble substances after meals. Consequently, temporary storage of bile is accomplished by contraction of the sphincter of Oddi, which prevents it from entering the duodenum and diverts it into the gallbladder. The amount of bile stored depends on the capacity of the organ.

Expulsion of Bile. Gallbladder contractions which expel bile can be demonstrated by three means: (1) direct observation on animals [2202] and man; (2) cholecystography [365, 366, 367, 1243]; (3) cholangiography in the presence of a biliary fistula.

TYPES OF CONTRACTIONS. Rhythmic contractions (tonus rhythm) occur spontaneously two to six times per minute [1603], even in gallbladder



FIG. 42 A. Cholecystogram 14 hours following the ingestion of 6.0 gm Telepaque. B. Appearance of the same gallbladder following a fat meal during the phase of contraction; the gallbladder rose in the abdomen and became more horizontal. The common duct is also faintly visualized. (A and B, courtesy of Dr. Emmanuel Salzman.) C. Cholangiogram showing termination of the common duct with reflux into the pancreatic duct. D. Cholangiogram showing spiral valve of Heister and dilated extrahepatic and intrahepatic bile ducts as a result of obstruction at the papilla of Vater. (C and D, courtesy of Dr. Lillian Donaldson.)

strips. When the cystic duct is closed, they slightly reduce the size of the gallbladder and elevate the intravesical pressure. Tonic contractions of the gallbladder as a whole after various chologogic stimuli, especially food, reduce the size of the organ and result in expulsion of bile (Fig. 42A and B). The gallbladder is not completely emptied, but its lumen is reduced to approximately thumb size (Fig. 42). The maximal expulsive

pressure exerted by the gallbladder is 20 to 30 cm bile [1605]. The emptying of the gallbladder is intermittent and rarely complete, either in man or dogs, even as a result of a strong chologogic stimulus such as a fat meal.

PHASES OF CONTRACTION. The first phase of contraction starts immediately following the appearance of food in the duodenum and it lasts for only a very few minutes, during which time the gall-

bladder rises, stiffens, and becomes oval-shaped. Following this, the emptying process is interrupted for a few minutes (2-minute pause) [365, 366, 367], after which the principal period of discharge, lasting about 30 minutes, occurs. During this period, two-thirds of the gallbladder is emptied. The fundus contracts, and contraction rings may be seen in the body of the organ. After a second period of quiescence of 5 to 45 minutes, the gallbladder contractions are resumed, so that the total evacuation may normally take from 16 minutes to 5 or 6 hours [365, 366, 367]. Two variations to this basic pattern occur. In the first, the gallbladder relaxes initially and the sphincter closes. This persists for several minutes, and then normal emptying proceeds. In the other type, evacuation is intermittent over several hours, with alternating partial emptying and refilling.

SPONTANEOUS CONTRACTIONS. The most important type of contraction is stimulated by food. Spontaneous gallbladder contraction owing to a stretch stimulus without the stimulating effects of food takes many hours and occurs after elimination of fat and protein from the diet. Even the smell of food leads to some evacuation. Hunger contractions and the siphon effect of the common duct with relaxation of the sphincter of Oddi have been named as factors responsible for spontaneous gallbladder emptying.

PHYSIOLOGIC VARIATIONS. The gallbladders of women empty faster than those of men; before puberty, however, the reverse is true [365, 366, 367]. Pregnancy in guinea pigs [3240] reduces the evacuation of the gallbladder after administration of the stimulating hormone, cholecystikinin (see Hormonal Stimulation, later in this chapter). In pregnant women, slow gallbladder evacuation is caused by reflex hypertonicity of the sphincter of Oddi, reflex inhibition of the gallbladder, sex hormone influence on the gallbladder, or increased viscosity of bile as a result of inhibition of bile secretion or increased excretion of solids [1604].

ABNORMAL VARIATIONS. An overdistended or very contracted gallbladder does not respond to normal stimuli, while a relaxed large gallbladder contracts well [365, 366, 367]. In addition to muscular contraction, elastic recoil from elastic fibers and membranes and from muscle aids contraction, especially in distended gallbladders. Laparotomy does not interfere with gallbladder contraction except for some distension for 48 hours. Peptic ulcers may either increase or decrease the gallbladder emptying time. Failure or retardation

of evacuation may be caused by hypertonicity of the sphincter of Oddi, inflammation of the ampulla, obstruction of the common duct, hypertonicity or inflammation in the duodenum, reversed peristalsis of the duodenum, diets containing inadequate cholagogic stimuli, or alterations of the cystic duct such as kinking by inflammation or obstruction by stones [1604]. The musculature of the gallbladder becomes hypertrophic after prolonged hypertonicity.

FUNCTION OF CYSTIC DUCT. The material in the lumen of the gallbladder is expelled through the cystic duct, which thus serves as a channel, with flow in both directions. This unusual functional principle resulted in the hypothesis that the gallbladder is filled through the cystic duct and emptied through lymphatic or venous channels [1360]. In freshly removed gallbladders the heisterian valve has been claimed to prevent the outflow of bile. Recent chemical [1360] and radiologic studies demonstrated that the cystic duct permits bile flow in both directions. Whether the heisterian valve is actually a valve or is merely the result of overgrowth of cystic duct epithelium is questionable. Overgrowth of epithelium was offered as an explanation of other kinks in the gallbladder or the ducts, including the diverticulum-like outpouching referred to as the Phrygian cap [365, 366, 367].

Concentration. Since space is at a premium in the right upper quadrant of the abdomen, owing to the presence of liver, right kidney, and intestine, it is not possible for much bile to accumulate in the gallbladder. This crowding is avoided by concentration of bile. Water, bicarbonates, and chlorides are reabsorbed in the gallbladder, leaving the biliary substances such as bile pigment, bile acids, cholesterol, and calcium to be excreted in a concentrated form [1603, 1605]. The bile is concentrated about tenfold, but an even greater concentration of bilirubin can be noted, since the ratio of bilirubin in gallbladder and liver bilirubin varies from 0.96 to 24.5 [2037]. The resulting increase in color differentiates the gallbladder bile from the much lighter liver bile.

Reabsorption starts rapidly after the entrance of bile into the gallbladder, and progresses with time. In dogs a maximum is reached in about 24 hours, the gallbladder bile becoming saturated at a concentration of approximately 20 to 25 per cent solids [1603]. The concentrating function rests largely with the mucosa. Concentration is increased in the presence of papillomas or hyper-

trophic villi, which may develop after prolonged stasis.

ABSORPTION OF BILE ACIDS. Bile acids are not absorbed by the normal gallbladder but are rapidly absorbed by inflamed mucosa [1664], a hepatocystic circulation of bile acids. Under these circumstances, cholic acid is more rapidly absorbed than desoxycholic acid, while glycocholic acid is more rapidly absorbed than taurocholic or unconjugated bile acids. Comparison of the constitution of liver and gallbladder bile reflects not only the degree of stasis but also inflammation of the mucosa [817].

ABSORPTION OF CHOLESTEROL. Absorption of cholesterol [108] and secretion by the gallbladder have been claimed, but the consensus is that cholesterol is only concentrated with other biliary constituents [74]. Cholesterol has also been said to pass in or out through the gallbladder wall, depending on the relative blood and bile cholesterol levels [2037]. The range of the ratios of cholesterol in hepatic and gallbladder bile varies from 0.6 to 21.4 [2037], and the normal cholesterol/bile salt ratio is 1:20 to 1:30. Under abnormal circumstances cholesterol is absorbed on the tips of the folds of the gallbladder mucosa, with resulting deposition in its stroma, producing the strawberry gallbladder, or cholesterolosis. In inflammation or hemorrhage in the gallbladder, cholesterol is released into the lumen of the organ.

ABSORPTION OF ELECTROLYTES. Calcium is mainly concentrated by the normal gallbladder, although a small amount may be absorbed, since its concentration does not parallel that of other bile constituents. In the obstructed gallbladder, secretion of calcium contributes to its high concentration in the bile. Chloride and bicarbonate concentration result in a greater acidification of the gallbladder bile in comparison with liver bile [2721].

ABNORMAL ABSORPTION. Absorption of dyes such as those used in cholecystography occurs only under abnormal circumstances. After prolonged cystic duct obstruction, the gallbladder content is water-clear or it contains calcium carbonate (milk-of-calcium bile).

Secretion. The only substance the gallbladder is definitely known to secrete is mucin. The gallbladder contributes a considerable portion of the 20 ml mucin added daily to the bile. Because the various antimicrobial substances are not secreted, they are not effective in obstructive cholecystitis [3694].

Pressure Regulation. The pressure in the biliary system depends on the interplay of the back pressure exerted by the sphincter of Oddi and the secretory pressure of the liver. The gallbladder, acting as an expansile side arm of the biliary system, regulates the pressure in the ducts and prevents undue increases which might cause the clinical manifestations discussed. The gallbladder is developed in all animals that have a sphincter of Oddi, apparently to avoid undue pressure. Animals with high sphincter resistance, such as dogs, cats, mice, and chickens, have gallbladders with great concentrating power and a small output of bile, while rats and horses have neither a gallbladder nor a sphincter of Oddi but have a large output of bile. The role of the gallbladder is also illustrated by the observation that the serum-bilirubin level rises after ligation of the common duct much sooner in cholecystectomized dogs than in normal ones [2202].

Extrahepatic Bile Ducts

The common and hepatic ducts, if not dilated, contract rather slowly, and peristaltic waves can be demonstrated by roentgenologic observation [2115]. The extrahepatic biliary ducts seem to have tone, loss of which results in abnormal dilatation in the absence of obstruction at or above the papilla of Vater. Normally, secretion exceeds absorption in the ducts. If portions of the hepatic duct are isolated by obstruction, colorless viscid fluid accumulates.

Sphincter of Oddi

Four functions have been ascribed to the sphincter of Oddi [228].

1. Regulation of bile flow into the duodenum. The sphincter of Oddi regulates the discharge of bile into the duodenum by offering resistance to the flow of bile.

2. Prevention of regurgitation of duodenal contents into the bile ducts. The absence of the sphincter facilitates infection [2879], although internal biliary fistulas produce only mild degrees of infection. In animals without sphincters, other mechanisms such as valves or peristaltic movements prevent ascending infection.

3. Control of the filling of the gallbladder.

4. Erection of the papilla. Contraction of the sphincter of Oddi erects the papilla of Vater, thereby producing an ejaculatory movement which aids in the emptying of the gallbladder and biliary tract.

Under basal circumstances, the sphincter of Oddi is contracted and no bile enters the duodenum. If the gallbladder is filled to capacity, some liver bile trickles into the duodenum through the somewhat relaxed sphincter. This occurs only during fasting and not when regular meals cause contraction of the gallbladder at normal intervals. The sphincter of Oddi apparently functions independently of the duodenal muscle [228], although spasm of the duodenal muscle eventually causes contraction of the sphincter [2408].

The resistance offered by the sphincter of Oddi not only varies with respiration or because of factors temporarily increasing intraabdominal pressure, but even varies spontaneously. Reflex contraction by stimulation of the colonic nerves has been demonstrated [1605] and has been considered the reason for increased resistance in constipation and possibly also in pregnancy. The response of the sphincter to foods or drugs is discussed in connection with the gallbladder response. Abnormal spasms of the sphincter and their clinical significance are discussed under Biliary Dyskinesia, Chap. 30.

Interrelation between Gallbladder and Sphincter of Oddi

Contraction of the sphincter of Oddi synergistically acting with relaxation of the gallbladder is required for the filling of the bladder. The duodenal resistance acts synergistically with the pressure of the sphincter of Oddi, and the two can not be easily separated. Similarly, the prompt evacuation of both liver bile and the stored gallbladder bile upon entrance of food into the duodenum depends upon coordinated contraction of the gallbladder and relaxation of the sphincter of Oddi. According to Meltzer's law, the innervations of the gallbladder and the sphincter are antagonistic. If the sphincter of Oddi is surgically removed, the gallbladder contracts very little. This coordinated response is evoked not only by nervous stimuli, which have only a temporary effect, but also by humoral factors, which are usually more important. Therefore, the stimulating agents are discussed for both actions together, although many of them have a primary action only on either the sphincter of Oddi or the gallbladder.

HORMONAL STIMULATION. Some chemical substances release a "hormone" from the upper intestinal mucosa, "cholecystokinin" [1603], which causes contraction of the gallbladder. Denervation of the gallbladder does not prevent the normal

evacuation in response to food. Acid placed in the duodenum of a donor dog produces contractions of the gallbladder of a recipient dog in cross-circulation experiments. These observations, supplemented by additional experiments in many species, including man, support the existence of the hormone [1299]. Many substances, among which fat, dilute hydrochloric acid, and peptones are the most important, stimulate the release of cholecystokinin from the intestine [1603]. After intravenous injection, cholecystokinin rapidly produces the peak of contraction after 3 or 4 minutes, with a return to the original shape in 10 to 30 minutes. Smaller doses given repeatedly produce rhythmic, unsustained contractions which completely evacuate the gallbladder in 1 hour [1603]. Whether vagal activity mediates the formation and release of cholecystokinin is questionable [1299]. Since the preparations of cholecystokinin available cause increased duodenal contractility, studies of the action of this hormone on the sphincter of Oddi are difficult, but cholecystokinin supposedly relaxes it [1299].

NERVOUS STIMULATION. The gallbladder is innervated by sympathetic fibers from the splanchnic nerves and by parasympathetic fibers from the vagus nerves. In general, the splanchnic nerves are largely inhibitory, while the vagus fibers are stimulating [2202]. However, this typical nervous response of the gallbladder is not constant. Mild stimulation of the vagi relaxes the sphincter of Oddi, whereas stronger stimulation contracts it. This phenomenon has been explained by antagonistic innervation in different parts of the sphincter, in that the duodenal, or antral, part of the muscle system is contracted by vagal stimulation and relaxed by sympathetic stimulation, in contrast to the situation in the sphincter of Oddi itself. The contraction of the duodenal portion under strong vagal stimulation may thus prevent emptying of the gallbladder, in view of the closed duodenal part of the sphincter. Whether this mechanism, demonstrated in the experimental animal and considered important in the explanation of various abnormalities, holds true for man is not established [228].

Section of the splanchnic nerves produces little change, whereas section of the left vagus retards somewhat the gallbladder emptying. Section of the right vagus results in prolonged retardation of the emptying, because this nerve also influences the sphincter of Oddi [1645]. Complete denervation, however, fails to influence the emptying time

[367]. Following vagotomy for duodenal ulcer, the volume of bile in the gallbladder increases to twice normal, as a result of the loss of the vagal effect on tone, rather than on emptying; following vagotomy the incidence of biliary disease is not increased [1645].

Most emptying stimuli first produce a contraction of the gallbladder with a rise in intraductal pressure for about 2 minutes, the 2-minute pause

of Boyden [365, 366, 367], until the sphincter opens and permits the evacuation of the gallbladder. Spinal anesthesia decreases sphincter tone. Sympathectomy in animals causes experimental acute cholecystitis, and sympathetic block in man ameliorates its course by causing relaxation of the sphincter [1557]. Emotional factors such as fear or anger may produce gallbladder contractions, a fact best explained by vagal stimulation.

Table 6 Effect of Various Drugs on the Biliary Tract and on Bile Flow

Drug	Dose and route	Effect on sphincter	Effect on gall-bladder	Effect on biliary pressure	Effect on bile flow
Morphine.....	15 mg subcutaneous	Prompt and prolonged sphincter contraction	No effect or slight increase in tonus rhythm	Increased	Decreased
Codeine.....	60 mg subcutaneous	Same as morphine but less	No effect	Increased	Decreased
Pantopan.....	20 mg } subcutaneous	Same as morphine	No effect	Increased	Decreased
Dilaudid.....	2 mg }				
Amyl nitrate.....	Inhalation	Relaxation (except for spastic sphincter)	Relaxation	Decreased	
Nitroglycerin.....	0.5 mg sublingual	More prolonged relaxation but less than with amyl nitrate	Relaxation	Decreased	
Erythrol tetra-nitrate	60 mg oral	No effect	No effect	May be decreased	
Theophylline.....	250 mg intravenous	Questionable relaxation	No effect	Probably no effect	
Papaverine.....	30 mg intravenous	Questionable relaxation	Relaxation	Reduced	Increased
Histamine.....	0.5 mg intramuscular	Some relaxation but contraction topically	Contraction		
Atropine.....	0.5-1.0 mg intramuscular	Unchanged; relaxation in some animals	Slight decrease in tonus rhythm	Unchanged	May be increased
Trasentine.....	75 mg intramuscular	Some relaxation	Relaxation	No effect	
Ethyl alcohol.....	30 ml oral	Occasional relaxation		No effect	
Pilocarpine.....	6 mg intramuscular	Contraction, especially in animals	Contraction	Increased	Decreased
Prostigmine 1:4,000	1 ml intramuscular	Variable	No effect	Variable	Decreased; then increased
Physostigmine.....	100 mg intramuscular	Variable	No effect	Variable	Decreased; then increased
Acetylcholine.....	2.5 mg intramuscular	Some contraction	No effect	Occasionally decreased	
Mecholyl.....	15-20 mg intramuscular	Contraction		No effect	
Epinephrine 1:1,000	0.5 ml intramuscular	Variable	Variable	Variable	
Ephedrine.....	50 mg intramuscular	Same as epinephrine	Same as epinephrine	Less than epinephrine	
Propadrine.....	20 mg in dog intramuscular	Same as epinephrine	Same as epinephrine	Less than epinephrine	
Benzedrine.....	20-30 mg oral	Relaxation in animals; no effect in man	Relaxation	Decreased	
Caffeine sodium benzoate	0.75 gm intramuscular	No change	No change	No change	
Hydrochloric acid or gastric juice	0.4-0.9% intraduodenal	Variable, usually contraction	Variable	Usually increased	May be stimulated
Calcium chloride...	1.0 gm intravenous	Variable	May increase tonus rhythm and evacuation	No effect	Occasionally increased
Posterior pituitary (pituitrin)	1.0 ml intramuscular	Variable in dogs; no effect in man	Some contraction	Moderately increased	
Bile salts.....	Oral or intravenous	Variable	Variable	Variable	
Isotonic saline.....	200 ml in dog intravenous	Contraction	No effect	Increased	Decreased
Hypertonic saline...	10 ml in dog intravenous	Contraction	Relaxation		Not changed
Glucose, 5%.....	200 ml in dog intravenous	Moderate contraction	Variable		Increased
Glucose in isotonic saline	200 ml in dog intravenous	Variable	Slight contraction		Increased
Insulin.....					Increased
Nicotine.....	Intramuscular	Contraction	Relaxation		Increased
Ergotamine 1:2,000	0.5 ml intramuscular	No effect	Slight contraction		Increased
Magnesium sulfate	1 gm intraduodenal	Relaxation			Increased
Phosphorantonic...	120 mg intramuscular	No effect	No effect	No effect	

STIMULATION BY FOODS. Foods usually produce simultaneous contraction of the gallbladder and relaxation of the sphincter. The effects on the sphincter have been demonstrated in patients with biliary fistulas [228, 817], while effects on the gallbladder are based mainly upon roentgenologic investigations (Fig. 42A and B). Egg yolk and cream provide the strongest stimuli of all foods, a mixture of both being even more effective, especially for causing relaxation of the sphincter. This is followed by olive oil and then by meat proteins and peptones. Carbohydrates are without effect upon the sphincter tone or gallbladder contractibility. Despite the antagonistic reaction, egg yolk, for instance, may first produce a transient spasm of the sphincter of Oddi before relaxation occurs, thus causing the 2-minute pause [365, 366, 367]. During this time the gallbladder contracts against a spastic sphincter of Oddi, and no bile flows. The independence of the response of the gallbladder and sphincter of Oddi to the same stimulus is also indicated by the remaining activity of the sphincter after cholecystectomy [228].

CHOLEKINETIC DRUGS. Pharmacologic stimuli follow Meltzer's law, as a rule, by antagonistically influencing the gallbladder and sphincter of Oddi. The agents which produce gallbladder contractions and simultaneous relaxation of the sphincter are called "cholekinetic" or "cholagogic" agents. They act either via the autonomic nervous system or via cholecystokinin. Intraduodenal magnesium sulfate, slightly inferior in effect to egg yolk, causes an average decrease of 42 per cent of the gallbladder volume and lowers sphincter resistance about 3.0 cm in 30 minutes, in contrast to egg yolk, which reduces the gallbladder volume 71 per cent and the sphincter resistance 7.0 cm in the same period of time [229, 366]. The effect is apparently mediated through cholecystokinin. Histamine stimulates smooth muscle in general and contracts the gallbladder, while occasionally relaxing the sphincter, the end result being a cholekinetic effect [228].

Most other drugs studied do not produce a synergistic relaxation of the sphincter of Oddi and contraction of the gallbladder. Antagonistic effects are noted, and the response reported varies considerably, depending upon the size of the dose. Table 6 presents a collection of data based on the experiences of several authors [228, 1850]. Pilocarpine produces a spasm of the sphincter of Oddi simultaneously with a contraction of the gallbladder; atropine relieves this.

EFFECT OF CHOLECYSTECTOMY. After cholecystectomy, the sphincter of Oddi usually becomes incontinent, as it does following spontaneous exclusion of the gallbladder by cystic duct occlusion [1666]. This incompetence is apparently not complete [1605], and eventually the sphincter recovers its tonus, sometimes with a dilatation of the intrahepatic and extrahepatic bile ducts, as demonstrated in dogs and in man [229]. In keeping with the dilatation of the ducts, the serum-alkaline phosphatase activity may be increased [834]. The dilatation is apparently an expression of the acceptance of the storage and reabsorptive functions of the gallbladder by the bile ducts. The lack of pressure-regulating activity of the gallbladder is probably important, although when a diseased gallbladder is removed it has already lost its pressure-regulating abilities. Gallbladder removal does not necessarily interfere with intestinal functions, such as fat absorption. After cholecystectomy, the attempt should be made to obtain a thin, freely flowing bile; this is best accomplished by frequent protein feedings or by administration of bile acids.

REFLUX BETWEEN PANCREATIC AND BILIARY DUCTAL SYSTEMS. The anatomic structure of the papilla of Vater determines whether spasm of the sphincter of Oddi facilitates reflux of either biliary contents into the pancreatic ductal system or pancreatic juice into the biliary system. Whether such a reflux actually takes place is problematic even in the presence of a functional communication, and if it does, whether it has clinical significance is not known. Reflux of pancreatic juice into the gallbladder has been considered proved by the demonstration of pancreatic enzymes in the gallbladder bile [817, 2653]. The filling of the pancreatic duct by regurgitated bile is seen in cholangiograms (Fig. 42C) and has been associated with pancreatitis. Much bile has been found in the pancreatic duct, however, without pathologic consequences [2280].

Regeneration of Bile Ductules and Ducts

"Ductular Proliferations." The so-called "proliferations" of bile ducts (Fig. 30E) are encountered in many hepatic disorders. They are usually seen in the periportal zone of the parenchyma but may also occur within the portal tracts, and are sometimes found deep within the parenchyma. Many are found in periportal inflammation and in areas of necrosis, and sometimes they are present even without severe tissue injury in cholestasis.

They resemble bile ducts but differ from typical bile ducts by showing basophilia and, occasionally, glycogen, fat, and bile pigment in the cytoplasm, by the presence of a cuticular membrane characteristic of bile canaliculi attached to the hepatic cells, and by the absence of a mucus layer stained with PAS. These structures are surrounded by a basement membrane, in contrast to the hepatic cells. Although the epithelial cells in these structures usually resemble those of ductules, in some places the differentiation from hepatic cells is difficult. Three-dimensional reconstructions by Elias of such structures indicate that some of them are tubules, but when they are numerous they usually are plates two cells in thickness. These plates form a wallwork similar to that of the hepatic cells. Between the plates abundant connective tissue is found. The tubules or plates secondarily connect with bile ducts.

ORIGIN OF "PROLIFERATED DUCTULES." Some authors consider all structures simulating proliferated bile ducts as derivatives of bile ducts or even remnants of preformed bile ducts in a collapsed area. Usually they assume that these "bile duct proliferates" in turn form hepatic cells. Mitotic figures on the tips of the buds connected with definite bile ducts are listed as evidence in favor of this assumption. Others deny this possibility [2188]. Herxheimer and his students [1470] consider these structures as proliferating or atrophic hepatic cells that eventually give rise to typical hepatic cells. They do not believe that hepatic cells develop from regular bile ducts or their proliferations.

Embryologically ductules develop from hepatic cells and not vice versa, especially if they are surrounded by connective tissue [1545]. The blood pressure in the surrounding veins seems to be the stimulus for further transformation of ductules into ducts (see Embryology, in Chap. 20).

Similar transformations occur in postnatal life as a result of abnormal stimuli. Three types of changes are seen:

1. Hepatic cells are apparently transformed into ductules and then into ducts especially when the numbers of ductules are increased within the lobule (Fig. 36, upper right). Transitional cells can be seen.

2. Degenerating hepatic cells and subsequently regenerated two-cell-thick plates sometimes resemble ductules or develop into them, especially in massive or submassive necrosis (Figs. 30E, 31, bottom, and 93, bottom).

3. The preexisting ductules and smallest bile ducts lengthen, resulting in tortuosity, especially on the lobular periphery (Figs. 31, bottom, 83C, and 92, top). They subsequently sprout, initially in the form of solid cords which become hollow tubes. These side arms are nonfunctioning structures, but a secondary connection with separated bile canaliculi in hepatic-cell plates may provide new channels for bile flow.

CAUSES OF "DUCTULAR PROLIFERATION." Structures resembling proliferated bile ducts seem to result from contact with connective tissue, humoral stimulation, biliary obstruction, or interruption of the bile duct system.

Contact with collagenous connective tissue produces ductular proliferations after necrosis or near the site of traumatic injuries to the liver. In transplantation experiments such structures arise from the edge of the transplant [327].

Humoral stimulation by growth factors affects the ductules, as it does the hepatic cells, after removal of part of the liver. In parabiotic, partially hepatectomized rats, the ductules appear to proliferate. This is also provoked by carcinogenic substances. One of the early manifestations of butter-yellow intoxication is ductular proliferation, including sprouting which extends far into the lobular parenchyma [2489]. A similar lesion can also be rapidly produced by chronic ethionine intoxication or other poisons which produce either necrosis or inflammation. Similar proliferations have been produced by partial occlusion of the bile duct by feeding a fat-free diet also deficient in vitamin A or by administration of thiourea [1171].

Biliary obstruction acts either mechanically or through growth factors contained in regurgitated biliary substances. The presence of these substances in the blood explains the proliferation of bile ducts and ductules in biliary obstruction. Proliferation can not be explained on a mechanical basis only; ligation of the common duct in one parabiotic twin causes bile duct regeneration in the other [2340].

Interruption of the bile duct system at the level of ductules [943], as in inflammation, is another potential factor in stimulating bile duct proliferation.

Regeneration of Bile Ducts. INTERLOBULAR BILE DUCTS. The factors listed above can also be applied to the interlobular bile ducts, but their proliferation is far less common. In animals, after ligation of the common bile duct, interlobular bile duct

proliferation may exceed ductular proliferations. Carcinogens and growth factors after loss of liver parenchyma are distinctly less stimulating to the interlobular ducts. Iron accumulation, as in hemochromatosis, is a strong stimulus for bile duct proliferation. In general the bile duct epithelium

has greater ability to survive injury than the hepatic epithelium.

LARGE BILE DUCTS. The larger bile ducts rarely regenerate. Hyperplasia and hypertrophy of the ductal mucosa have been described in mice as a result of estrogen treatment [1131].

The liver has a unique circulatory system because blood is brought to it by the hepatic artery as well as by the portal vein. Most of the blood comes from the portal vein, which drains the viscera of the peritoneal cavity. Blood leaves through the hepatic veins.

THE HEPATIC ARTERY

Extrahepatic Portion. The celiac axis originates from the aorta as a short trunk just above the origin of the superior mesenteric artery. In 55 per cent of persons it splits above the pancreas into the left gastric artery, the splenic artery, and the hepatic artery [2284]. The left gastric artery extends from the cardia downward along the lesser curvature of the stomach. The splenic artery extends along the upper border of the pancreas, to which it supplies branches to the hilus of the spleen. Before terminating in the spleen, it gives off the short gastric and left gastroepiploic arteries. The hepatic artery first gives off the gastroduodenal artery, which passes behind the first portion of the duodenum and divides into the superior pancreaticoduodenal artery and the right gastroepiploic artery. The hepatic artery then turns upward, giving off the supraduodenal and right gastric arteries, the latter following the lesser curvature of the stomach and anastomosing with the left gastric artery. As the hepatic artery approaches the liver, it divides into the right, middle, and left hepatic arteries, the middle one usually originating from the left branch [2284]. The cystic artery arises from the right hepatic artery in the cystic triangle of Calot. The cystic and right hepatic arteries are best separated from each other in this triangle, limited by the cystic duct, the hepatic duct, and the liver. The cystic artery

branches into an anterior twig to the peritoneal surface of the gallbladder and a posterior twig to the gallbladder bed. The two twigs communicate freely with each other (Fig. 43).

VARIATIONS. The distribution of the hepatic artery varies greatly [418, 2284, 3021]. In over 30 per cent of dissections one branch of the hepatic artery was aberrant, and in about 10 per cent of cases two branches were. A displaced artery has a course different from the usual one, and its ligation compromises the blood flow to the parenchyma supplied by the vessel. Ligation of an accessory artery which is additional to the usual vessel has no significant effect. A displaced common hepatic artery may originate from the gastroduodenal, left gastric, splenic, or even superior mesenteric arteries. In the last instance it passes through or behind the head of the pancreas, where it might be ligated during a pancreaticoduodenal resection (Fig. 44). Occasionally the common hepatic artery is very short or missing and the right and left hepatic arteries are long and may originate independently from the celiac axis. The right hepatic artery may be displaced, originating from the superior mesenteric artery, and may be ligated at its crossing of the cystic and common ducts during cholecystectomy. An accessory right hepatic artery, less common than a displaced one, may be found as a second artery in Calot's triangle. In more than 10 per cent of persons the right hepatic artery crosses the anterior aspect of the hepatic duct. A displaced or aberrant left hepatic artery may originate from the left gastric artery. An accessory left hepatic artery may also be derived from the right hepatic artery.

Variations of the cystic artery are more common than those of the hepatic arteries. These are best recognized by careful dissection of Calot's triangle.

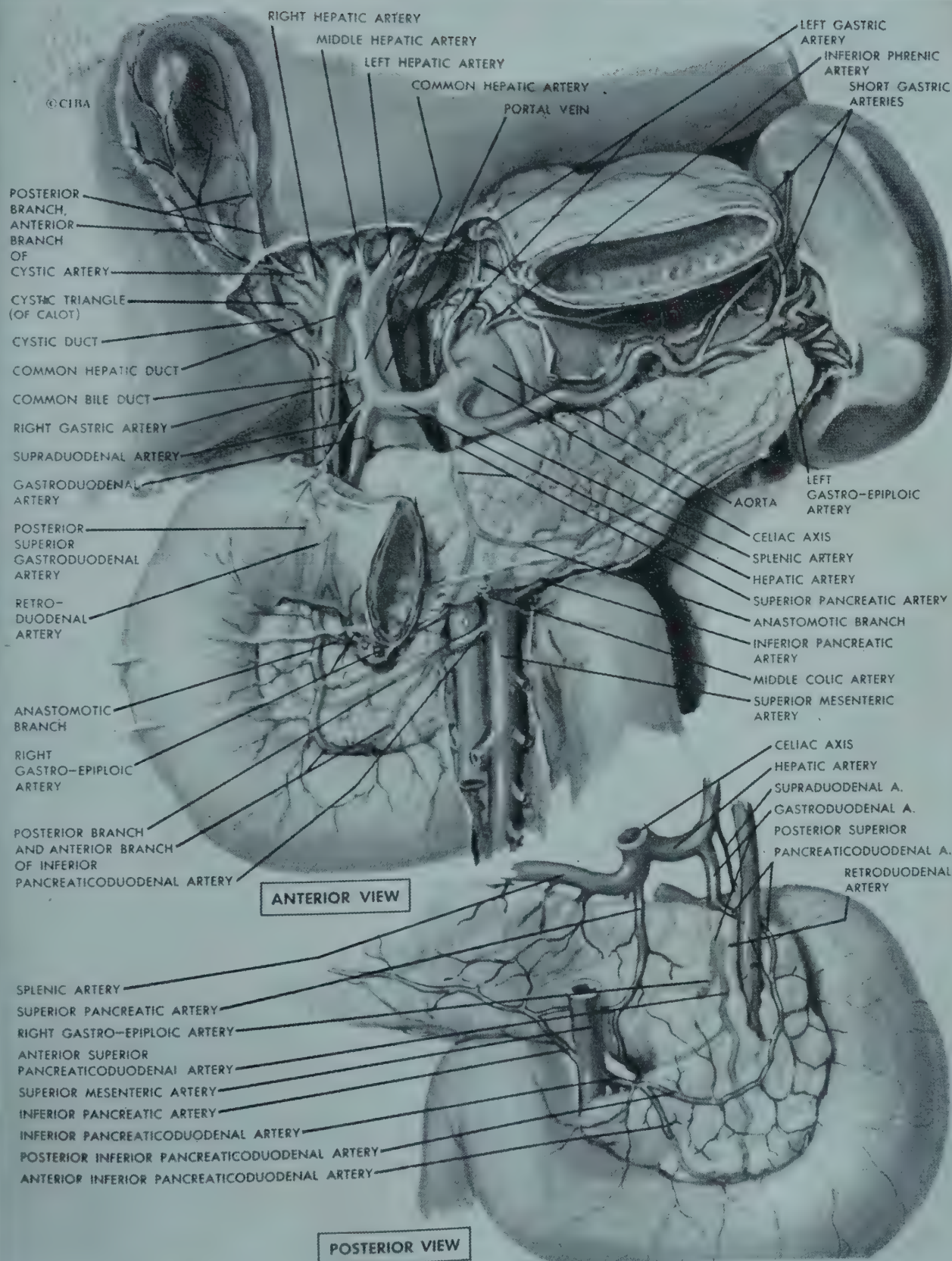


FIG. 43 The hepatic artery. (Copyright © Ciba Pharmaceutical Products, Inc.)

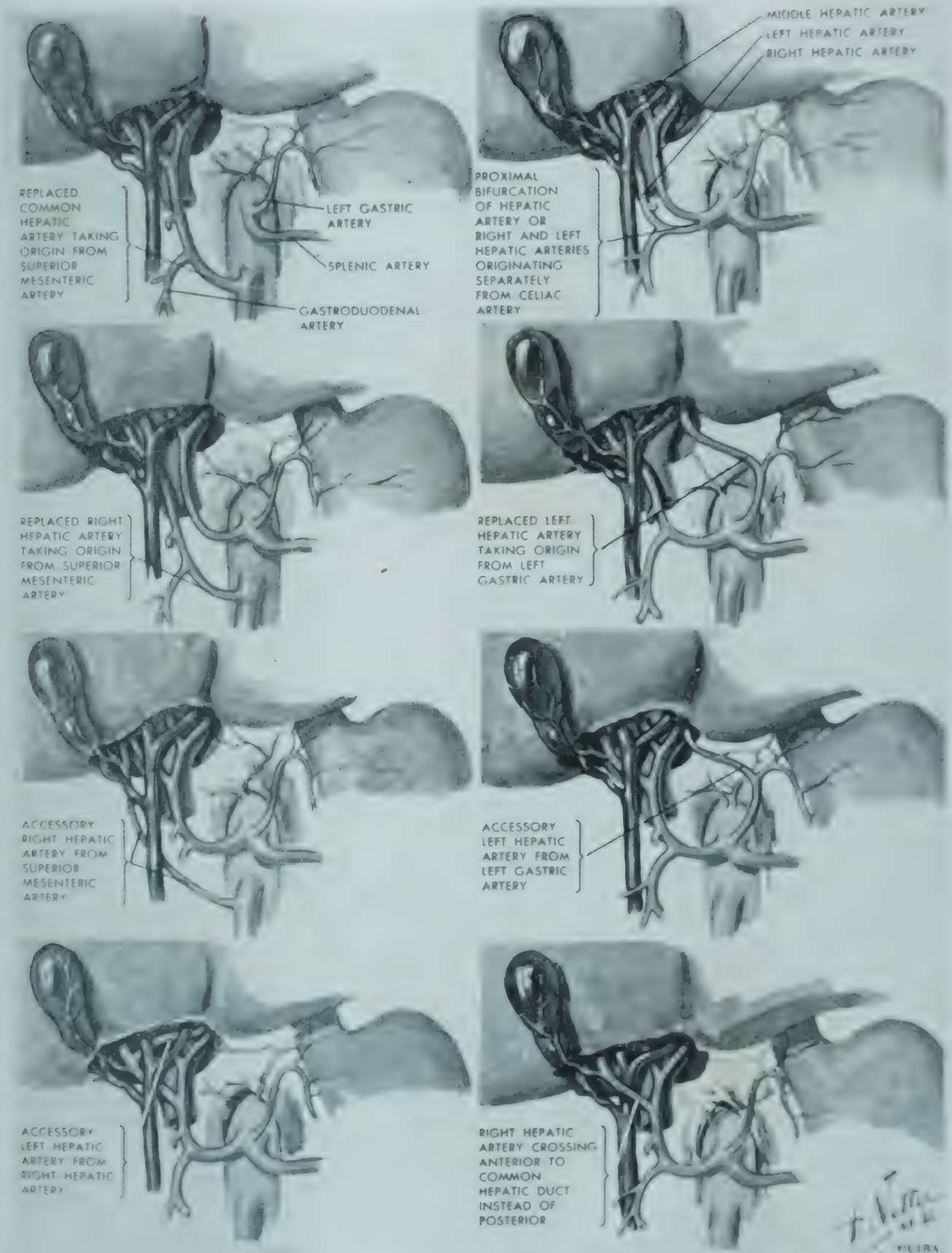


FIG. 44. Variations of the hepatic artery. (Copyright © The Ciba Collection of Medical Illustrations, Frank H. Netter, M.D.)

In more than 20 per cent of persons the cystic artery does not originate in the triangle but from the right, middle, or left hepatic arteries, the common hepatic artery, or even the gastroduodenal artery or the celiac axis. In these instances it crosses the hepatic or common bile ducts. Double cystic arteries occur in about one-fourth of persons, either both or at least one arising in Calot's triangle. The superficial or anterior artery usually originates below the posterior, or deeper, branch of the cystic artery.

Anastomoses of the hepatic artery with the phrenic artery lie in the layers of the peritoneum reflected from the liver onto the diaphragm and in the areolar tissue around the inferior vena cava and the falciform ligament. Generally, the aberrant branches of these anastomoses have practical importance, in that they may serve as collaterals when the hepatic artery or one of its main branches is obstructed by a pathologic process or by surgical ligation. This possibility is supposed to exist anatomically in 32 per cent of the cases. Branches which extend to Glisson's capsule also belong in this group. Their arteriolar ramifications communicate with the arterioles of the neighboring hepatic tissue.

Intrahepatic Portion. LARGER VESSELS. In the liver, the branches of the hepatic artery accompany the bile ducts and branches of the portal vein in the portal tracts and split in a dichotomous fashion [672, 909, 1438]. Whether intrahepatic communications exist between branches of the hepatic artery, and whether they are of functional significance are controversial questions on the basis of injection preparations [1187, 2220]. Surgical experiences after hepatic artery ligation favor the idea that the arteries in the major portion of the liver are end arteries. Some terminal branches, however, particularly subcapsular ones, anastomose with each other and form an arterial network in the capsule. These capsular branches also anastomose with branches of the phrenic, internal mammary, renal, and suprarenal arteries. In this limited sense and area, no end arteries are present. Individual differences play a great role in the functional significance of these anastomoses.

INTERLOBULAR ARTERIES (RADICULAR OR VAGINAL BRANCHES). In its final distribution, blood of the hepatic artery flows toward the portal tracts and the lobular parenchyma (Fig. 45). Fine branches of the hepatic artery supply a dense capillary network, the peribiliary plexus, in the connective tissue of the portal canals around the ves-

sels, bile ducts, and nerves (Fig. 46, upper). This capillary network is very close to the mucosal lining of the bile ducts and is drained by veins and venules into the portal vein or the parenchymal sinusoids. This anatomic principle gave rise to the concept of internal radicles of the portal vein and radicular portal veins (see The Portal Vein, later in this chapter).

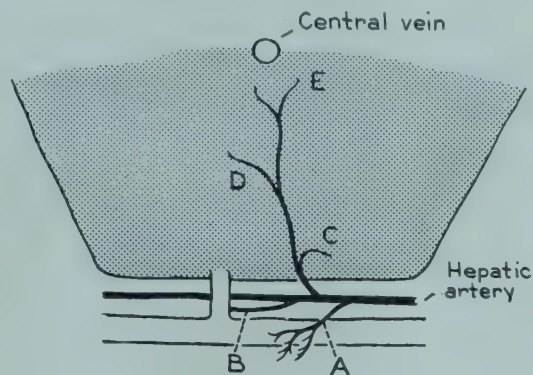


FIG. 45 Terminal intrahepatic distribution of the hepatic artery. A, radicular branches to peribiliary plexus; B, direct hepatic artery-portal vein communication; C, peripheral arteriole; D, mid-zonal arteriole; E, central arteriole. Direct communication between hepatic artery and portal vein, B, probably does not exist in man. Such communications appear in vital microscopy but seem to be caused by flow of blood from radicular arterial branches to the peribiliary plexus, A, and from there to the internal root of the portal vein (Fig. 49E).

INTRALOBULAR ARTERIOLES. Some arterial blood enters the sinusoids directly. Muscle pads in the terminal branches of the hepatic arteries regulate this blood flow and prevent eddy formation, which would occur when blood under high arterial pressure mixes with blood under low venous pressure [669]. In man, sphincters can be seen histologically as muscular elastic pads on one side of the lumen of medium-sized vessels or as uniform rings in the smaller vessels [669, 2582]. Recently the terminal distribution of the hepatic artery has been thoroughly studied [907, 909], and earlier observations have been confirmed. In injection preparations the hepatic artery was found to supply the central portion [3008] and the peripheral portion of the lobule (Fig. 46, bottom). Intra-lobular branches of the hepatic artery, "arterial sinusoids" which have sphincters, have been demonstrated in intravital microscopic studies [1808, 3463]. They are almost devoid of muscular elements but are still contractile, owing to the pres-

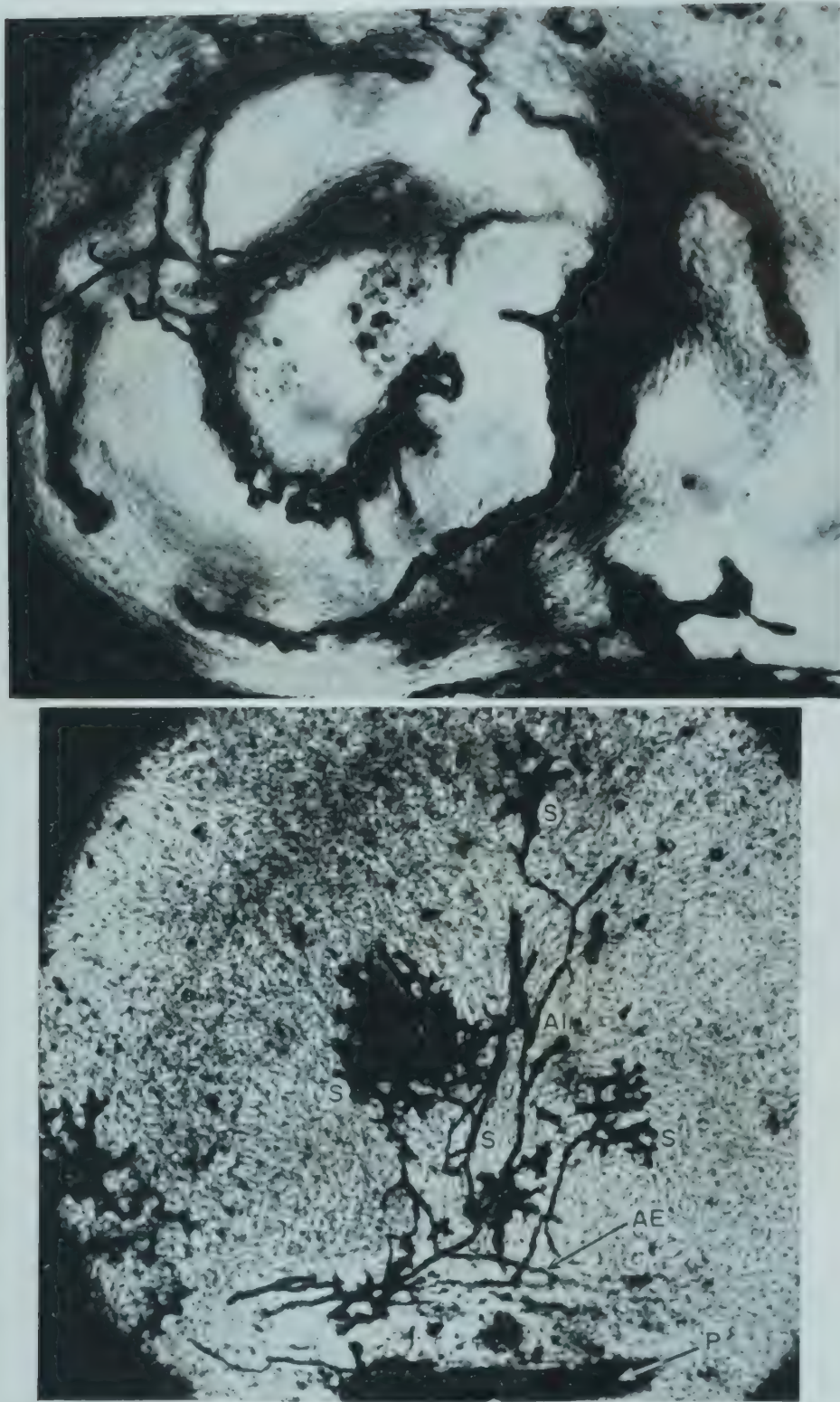


FIG. 46 Liver of a young man killed in an accident, injected with india ink through the hepatic artery. *Upper.* Cross section of medium-sized bile duct showing the subepithelial and submucosal plexus. The two plexuses are connected. (From original of Fig. 2, Elias, H., and Petty, D.: *Anat. Rec.* 116:17, 1953.) *Lower.* Longitudinal section of portal canal (below and of internal arterial vessels: AI, intralobular arterial capillaries, or arterioles; P, portal vein; S, sinusoids receiving direct arterial blood supply at all levels of the lobule; AE, extralobular arterial vessels. (From original of Fig. 4, Elias, H., and Petty, D.: *Anat. Rec.* 116:17, 1953.)

ence of pericytes. In three-dimensional reconstructions the course of arterial capillaries and arterioles was followed for a considerable distance into the lobule [907]. These arterioles, or arterial capillaries, are accompanied by bile ductules in fine connective tissue septums (Fig. 35). The state of contraction of these arterioles seems to determine whether arterial blood reaches the peripheral or the central portion of the lobule, or both.

THE PORTAL VEIN

Extrahepatic Portion. The portal vein usually forms behind the head and neck of the pancreas at the level of the second lumbar vertebra by union of the superior mesenteric and splenic veins (Fig. 47). The superior mesenteric vein forms at the mesenteric root mainly from the ileocolic, right colic, middle colic, and the inferior pancreaticoduodenal veins. The splenic vein starts at the hilus of the spleen and is joined by the left gastroepiploic, short gastric, pancreatic, and inferior mesenteric veins, the last shortly before it enters the portal vein. Variations of this typical distribution are found in almost 50 per cent of persons [819, 1169, 2674] (Fig. 48). The inferior mesenteric vein may enter the junction of the splenic and superior mesenteric veins, and in about one-fourth of all persons it enters the superior mesenteric vein.

The portal vein varies between 5.5 and 8.0 cm in length, with an average of 6.5 cm. It is normally about 1 cm in diameter, although this increases in cirrhosis. In about 90 per cent of persons, several veins enter the portal vein and these are readily lacerated during exploration prior to portocaval anastomoses. The coronary vein forms from the left gastric vein and the esophageal plexus and is of importance in supplying esophageal varices. In two-thirds of persons it enters the left aspect of the portal vein below the pyloric vein, which is a continuation of the right gastric vein. The coronary vein connects with the pyloric vein to form a loop. The coronary vein may also enter the junction of the splenic and superior mesenteric veins or the splenic vein itself. The right aspect of the portal vein admits the superior pancreaticoduodenal vein and the cystic veins, which are not uniformly developed and which may enter the right branch of the portal vein at the hilus of the liver. The upper 5 cm of the portal vein frequently has no major tributaries. No valves are present throughout the entire portal venous system. The

portal vein runs into the hepatoduodenal ligament to the hilus of the liver, where it splits into right and left branches, which extend with further subdivisions into the hepatic parenchyma along the bile ducts and the tributaries of the hepatic artery. The left main branch receives veins from the paraumbilical veins and exceptionally a persisting umbilical vein coming from the umbilicus in the round ligament (see Fetal Circulation, under Embryology, in Chap. 20).

Accessory Portal Veins and Anomalies of the Portal Vein. Small veins on the serosal surface of the liver and the surrounding peritoneal folds from the diaphragm and stomach are called "accessory" portal veins. They either unite with the portal vein or independently enter the hepatic parenchyma. The paraumbilical veins, communications between the portal system and the veins of the abdominal wall, may also function as accessory portal veins.

Anomalies of the portal vein are extremely rare (Fig. 48). Complete absence of the portal vein and its intrahepatic branches has been reported, with hyperplasia of the hepatic artery, a situation similar to that created by the construction of an Eck fistula [1452]. The portal vein may enter the renal segment of the inferior vena cava, in which case an enlarged hepatic artery supplies all the blood to the liver [565]. The umbilical vein was found to bypass the liver in one instance and enter the heart directly; the liver, however, developed normally. Some anomalies are of surgical importance. For instance, the portal vein and all its branches may be anterior to the duodenum and the head of the pancreas. Many small vessels may substitute for one portal vein. The pulmonary veins have been found to enter the portal vein. Strictures of the portal vein and persistence of the umbilical vein are described under portal hypertension (see Portal Hypertension, Chap. 29). Arteriovenous fistulas involving the portal vein and the hepatic artery cause portal hypertension [3250].

Intrahepatic Portion. INTERLOBULAR VEINS. The knowledge of the intrahepatic ramifications of the portal veins is based on the classic studies of Mall [2186]. Each branch terminally divides into two (dichotomous division), and the final terminal branches are the interlobular veins (Fig. 49). The larger interlobular veins, down to 400 μ in diameter, do not give rise to venules extending into the parenchyma but represent conducting veins [907]. The walls of these veins are composed of dense collagenous connective tissue, which merges grad-

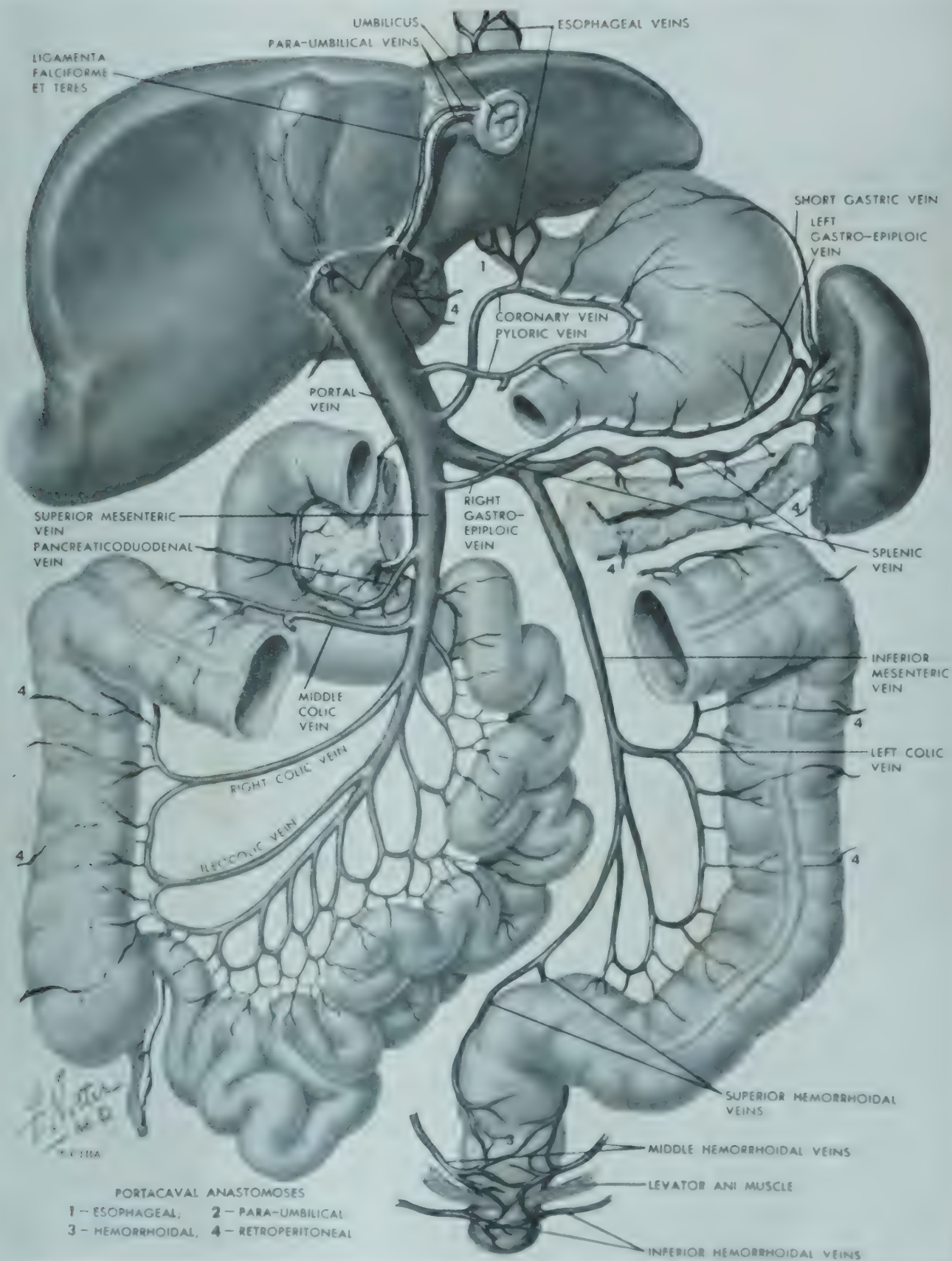


FIG. 47 The portal vein and its tributaries. (Copyright © The Ciba Collection of Medical Illustrations, Frank H. Netter, M.D.)

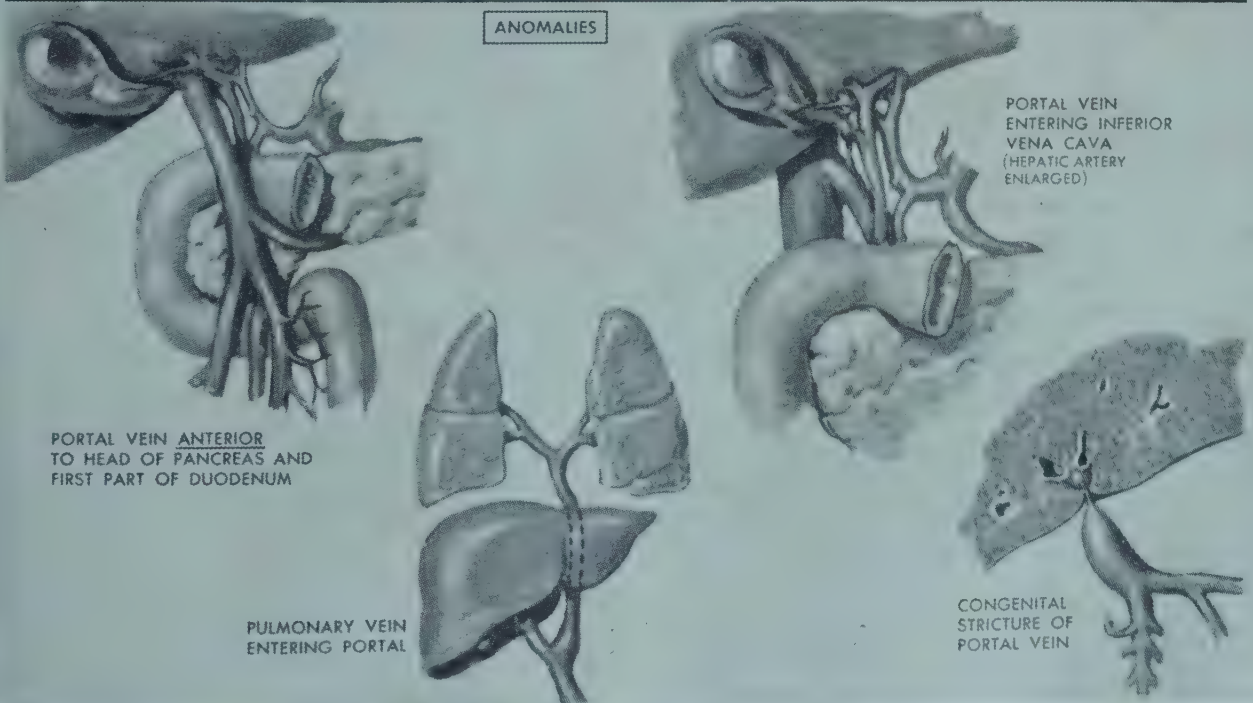
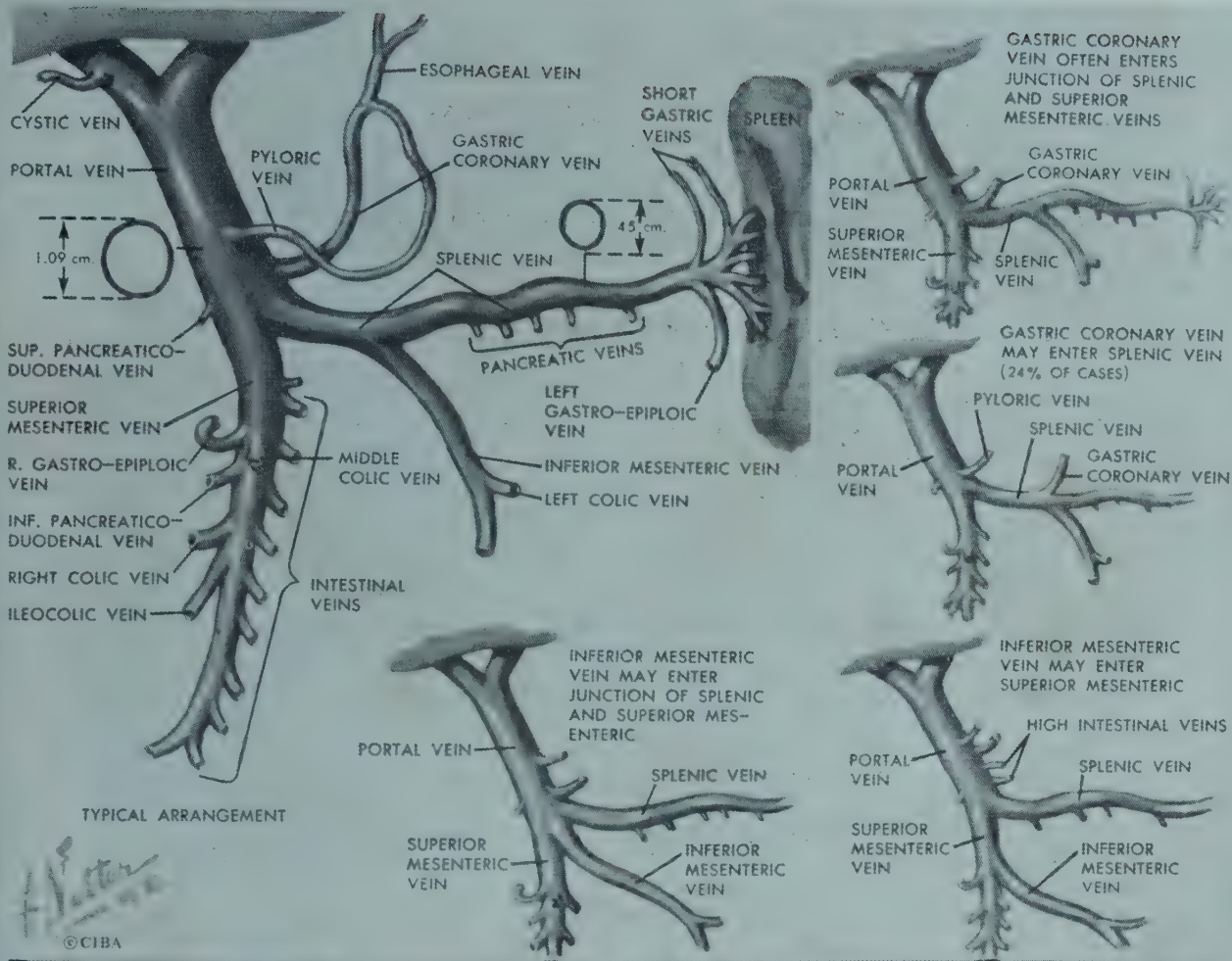


FIG. 48 Variation and anomalies of the portal vein. (Copyright © The Ciba Collection of Medical Illustrations, Frank H. Netter, M.D.)

usually with the connective tissue of the portal tracts. They are enforced by an elastic membrane and sparse muscle fibers, which fail to form a continuous layer. The conducting veins give rise to distributing veins, which may run alongside the larger conducting veins for a short distance. The distributing veins are almost identical in structure with the conducting veins except that they are thinner, and muscle fibers are even rarer.

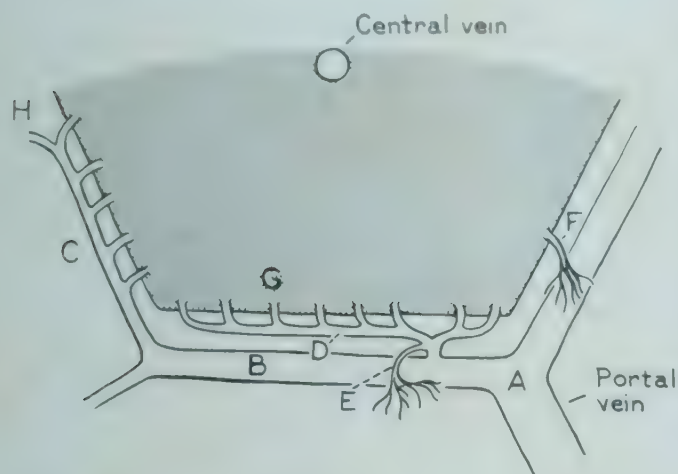


FIG. 49 Terminal distribution of the portal vein. A, dichotomic division of portal vein; B, conducting vein; C, axial distributing vein; D, distributing vein accompanying conducting vein; E, internal root of portal vein from peribiliary plexus in portal canal; F, radicular portal venule, from peribiliary plexus; G, inlet venule; H, terminal twig.

VENULES. From the distributing veins, smaller venules extend into the lobular parenchyma either at right angles as inlet venules or through terminal twigs, which usually arise on the border of two lobules. The twigs may extend into the fused area of both lobules, or into separate lobules with additional sinusoidal communications between the lobules. Most of the portal blood of the lobules is derived from the inlet venules, which are short trunks entering the lobule through the limiting membrane and then splitting in a treelike fashion into sinusoids. Two general types of sinusoids are described: peripheral sinusoids, which parallel the limiting membrane; and radial sinusoids, apparently emerging from the peripheral sinusoids and extending in a rather regular fashion toward the central vein (Fig. 50). Extensive communications between the sinusoids in all parts of the lobule obscure the basic pattern of sinusoidal distribution. In frogs, sphincters in the inlet venules on the borders of the portal tracts, interposed between

the terminal branches of the portal vein and the sinusoids, have been demonstrated; they define the pattern of arborization of the sinusoids. Variations in the size of the sinusoids depend on their state of contraction. In the larger vessels no sphincters exist, largely because the dichotomous branching does not lend itself well to sphincter action, although muscular elastic valves have been demonstrated [669].

VENOUS DRAINAGE OF THE PERIBILIARY PLEXUS. The venous return from the peribiliary plexus flows via one of two routes, the internal radicles of the portal vein, and the radicular portal veins.

Internal Radicles of the Portal Vein. The peribiliary capillaries unite to form small veins, which enter small intrahepatic branches of the portal vein. The existence of these branches has been denied [909], but recently injection preparations demonstrated large internal radicles of the portal vein draining hepatic carcinomas or areas of excessive bile duct proliferation around parasites (*Clonorchis sinensis*) in the absence of cirrhosis [2117].

Radicular Portal Veins. The small veins formed from the peribiliary plexus may drain directly into the parenchymal sinusoids and thus act as a miniature portal vein.

Portosystemic Anastomoses. The portal system normally communicates with branches of the vena cava, permitting small amounts of blood to bypass the liver. Normally such anastomoses are small and functionally insignificant [2164]. Under abnormal circumstances in portal hypertension or less frequently in inferior vena caval obstruction, they develop into major collateral blood channels. The clinically significant facts about these channels are (1) that they are visible and suggest the presence of portal hypertension; (2) that they bleed, especially those in the esophagus; (3) that they provide a bypass around the liver of blood from the intestine and thus contribute to the systemic effects of cirrhosis.

ANOMALIES. Abnormal terminations of the portal vein or its branches (see Congenital Anomalies of the Portal Vein, under Portal Hypertension, Chap. 29) and accessory portal veins, which may arise either from portal territories, such as the cystic, hilar, or lesser omental veins, or from systemic territories, such as the hepatorenal ligament or the diaphragm, should be differentiated from the actual portosystemic communications [890]. These accessory veins may also come from the inner layer



FIG. 50 Inlet venules arising from a portal vein branch and supplying the hepatic sinusoids. India ink injection into mesenteric vein of a living rabbit. (Elias, H.: *Liver Injury*, Tr. Eleventh Conf., Josiah Macy, Jr. Foundation, 1953.)

of the abdominal wall and communicate with the superficial vessels of the abdominal wall. Large congenital communications between portal and systemic vessels 1 cm or more in diameter are more common and are (1) splenorenal anastomoses; (2) persistence of the umbilical vein; (3) anomalous dilatation of the paraumbilical veins accompanying the umbilical vein in the round ligament and anastomosing with the anterior parietal vessels.

NORMAL COMMUNICATIONS. The normal communications may be divided into the anterior or parietal pathways and the deep pathways, which include the retroperitoneal, rectal, diaphragmatic, and esophageal branches. Study of specimens after injection with radiopaque material reveals that the deep communications normally are multiple fine channels but that occasionally they may be as large as 3 mm in diameter. Normally, the anterior channels seem to have less significance, but they readily enlarge as the result of obstruction. Since most of these communicating vessels have no valves, flow of blood is possible in either direction.

The anterior abdominal and the esophageal collaterals extend to the superior vena cava, while the remainder of the abdominal, retroperitoneal, and the hemorrhoidal collaterals enter the inferior vena cava (Fig. 51).

UMBILICAL VEIN AND PARAUMBILICAL VEINS OF SAPPEY. These veins run in the round ligament and connect the left branch of the portal vein with the deep and superficial abdominal veins, i.e., the inferior and superior epigastric veins. These in turn communicate with the mammary, intercostal, and costoaxillary veins. The superficial veins also communicate with the femoral and long thoracic veins. In portal vein obstruction these vessels become enlarged, particularly after the development of ascites, even in the presence of dilated deep collaterals. This enlargement is recognized clinically as the caput medusae, a radiating periumbilical dilatation. These veins in the abdominal wall can be differentiated from the more laterally arranged collaterals resulting from either superior or inferior vena cava obstruction. Infra-red photography is particularly useful in demon-

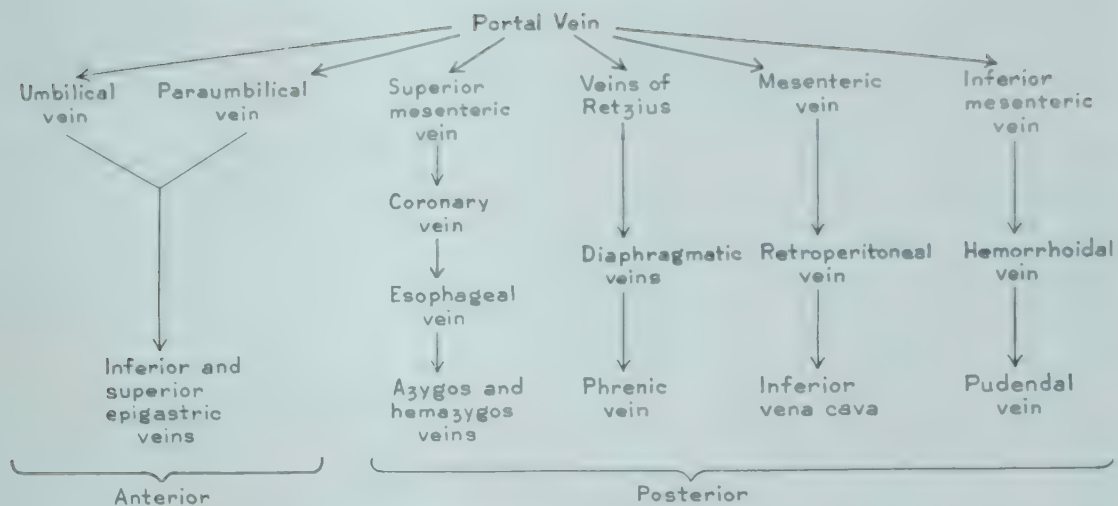


FIG. 51 Portosystemic communications.

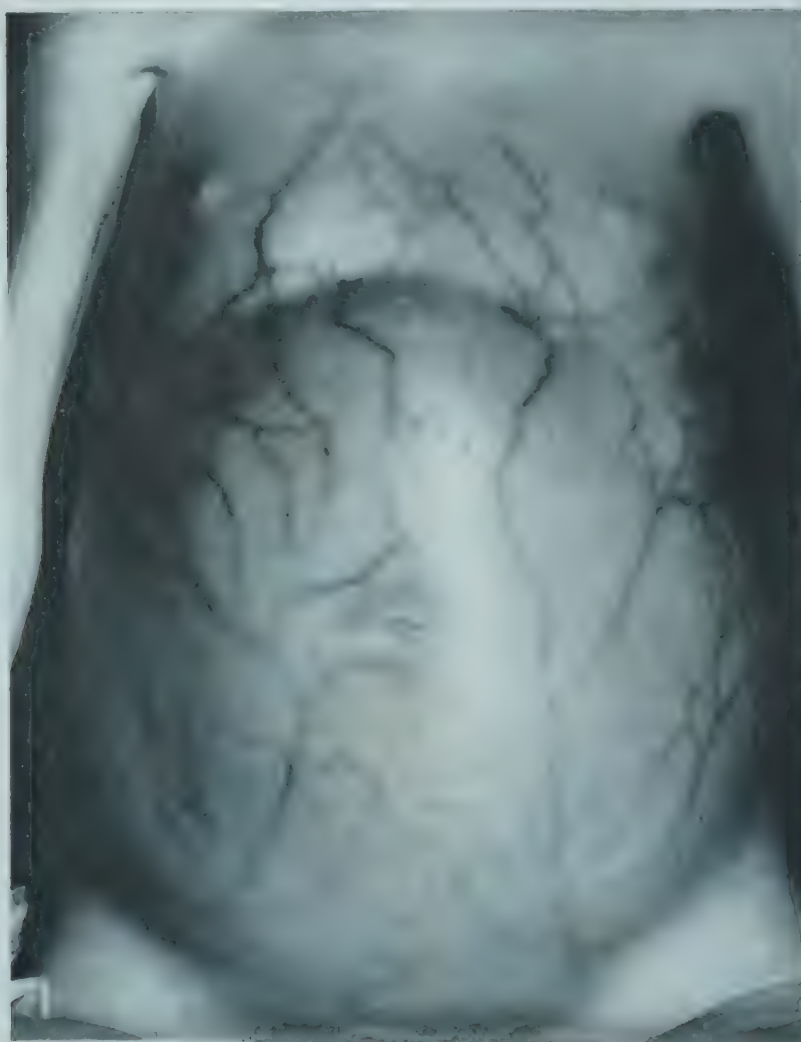


FIG. 52 Dilated abdominal veins in cirrhosis, demonstrated by infrared photograph.

strating these veins, which are often invisible upon ordinary inspection (Fig. 52). The function of these collaterals as carriers of portal blood is indicated by the higher concentration of ingested and digested foodstuffs in the blood of these veins in comparison with that in systemic veins [312, 3045], as well as by pressure readings in these vessels [733].

A special variety of this form with an excessive varicose dilatation of the superficial and deep abdominal veins is Cruveilhier-Baumgarten disease, which is also characterized by a bruit [99] (see Causes of Portal Hypertension, Chap. 29).

RETROPERITONEAL VEINS. The retroperitoneal veins, found chiefly around the kidney, are collaterals between the venous network of the peritoneum, draining into the superior and inferior mesenteric veins, and the retroperitoneal veins, which drain into the inferior vena cava. They dilate not only in portal hypertension but also in obstruction of the inferior vena cava, when they carry blood from the renal vein into the portal system [890, 3144].

HEMORRHOIDAL VEINS. Collaterals between the superior hemorrhoidal veins, draining into the inferior mesenteric vein, and the inferior hemorrhoidal veins, draining into the pudendal vein, may become enlarged and lead to formation of external or internal hemorrhoids.

ESOPHAGEAL AND DIAPHRAGMATIC VEINS. Esophageal varices are dilated communications between the veins draining the cardiac portion of the stomach (especially the right gastric or coronary vein, and also the gastroepiploic vein, and the short gastric veins) and the esophageal veins [1535]. The esophageal veins drain into the superior vena cava through the azygos and hemiazygos veins. The veins of the midportion of the esophagus have many anastomoses with the pericardial veins, the posterior mediastinal veins, the intercostal and diaphragmatic veins, all of which combine to form the azygos and hemiazygos veins. The veins around the lower third of the esophagus and the veins of the upper fourth of the stomach dilate in collateral formation. A venous hum can often be heard over the chest or epigastrium as a result of these dilated veins. Because the esophageal veins are more readily exposed to injury than the veins of the cardia, esophageal bleeding is far more common. Hemorrhage from esophageal varices is the most significant sequela of portal hypertension (see Portal Hypertension, Chap. 29). The diaphragmatic veins of Retzius are not of

great importance, but they dilate also as a result of portal hypertension.

THE HEPATIC VEIN

Intrahepatic Portion. The smallest branches of the hepatic vein start in the center of the lobules. These central veins have a thin wall composed of collagenous tissue with slight reinforcement by elastic fibers and membranes and only scattered muscle fibers, which have no definite arrangement. The connective tissue of the venous wall forms the limitation of the central field of the hepatic lobule. Several central veins join the sublobular or intercalated veins [907] at acute angles. These in turn unite to form the large collecting veins, which join to form the hepatic veins. The smaller veins receive tributaries throughout their entire length, with many small branches entering one wider vein, in contrast to the Y-type branching of the portal vein within the liver.

MICROSCOPIC ANATOMY. The microscopic structure of the intercalated, collecting, and hepatic veins is similar in principle to that of the central vein, except for an increase in thickness and number of muscle fibers. Continuous membranes or muscle bundles are absent in man. In man, small venules enter the central, intercalated, and collecting veins directly from the sinusoids. The intramural part of these sinusoids is merely an endothelial layer. The discrepancy between the small piercing vessel and the often relatively thick vein wall permits obstruction of the small vessel by moderate contraction of the larger vessel (Fig. 53, upper left). This obstruction is morphologically indicated by dilatation of the terminal portion of the sinusoids before piercing through the wall of the larger vessel [2625] (Fig. 53, upper right). Small sluice channels are formed from the confluence of many sinusoids and are seen in many animals [780]. At the entrance of the hepatic veins into the vena cava, reinforcement of the muscular layers produces a sphincterlike arrangement [912].

Extrahepatic Portion. The hepatic veins, of which there are several, empty into the inferior vena cava, where the latter runs adjacent to the posterior surface of the liver. The entrance is in the uppermost part of this portion of the vena cava directly below and on the level of the diaphragm. Individual variations may be of significance, especially in pericarditis [912]. The intrathoracic portion of the inferior vena cava is short

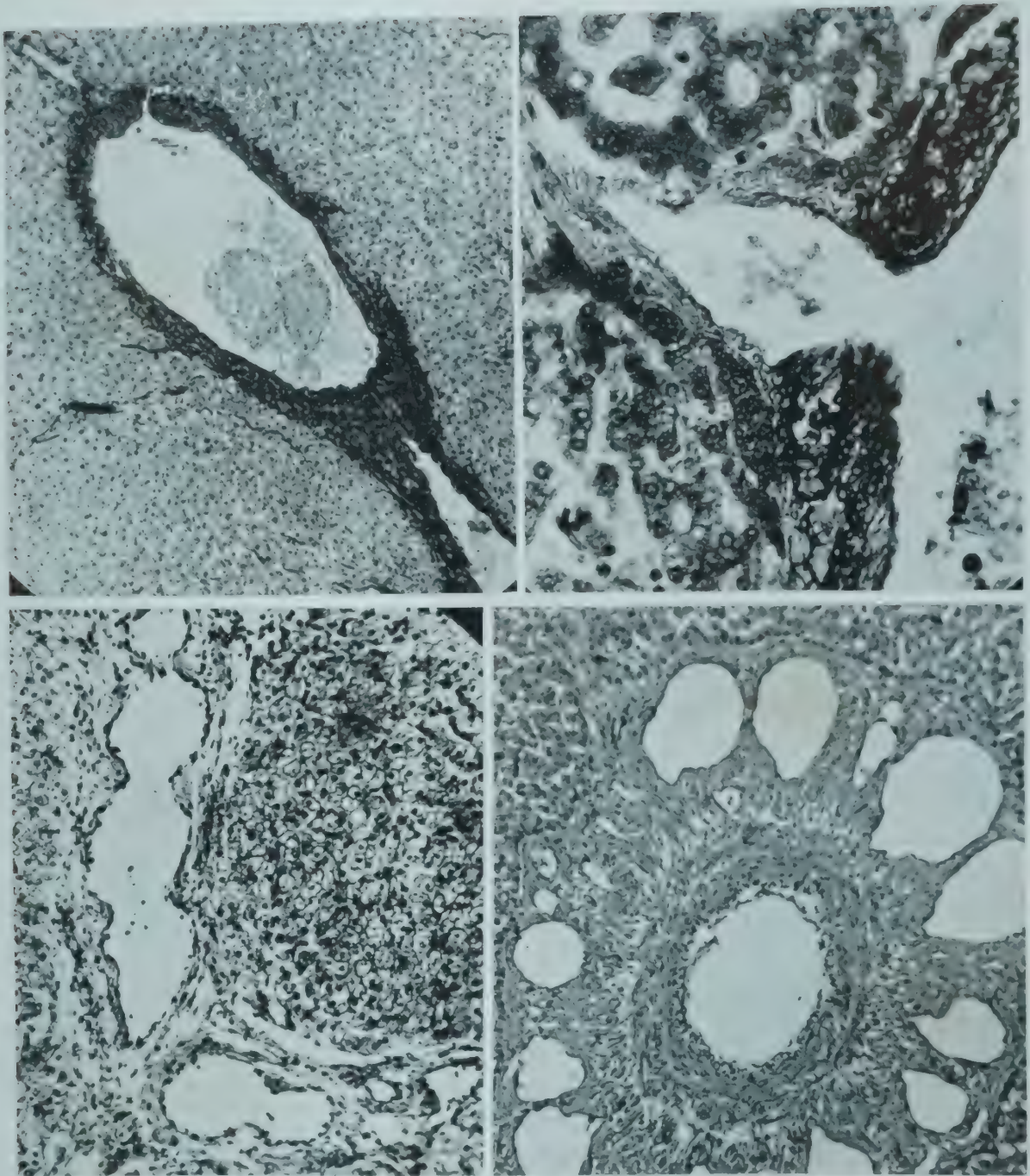


FIG. 53: *Upper left.* Contracted human sublobular vein with piercing central vein, revealing sinusoidal dilatation near entry. Mallory aniline blue ($\times 50$). *Upper right.* Narrowing of central vein where it passes into sublobular vein, with sinusoidal dilatation before entry. Mallory aniline blue ($\times 250$). *Lower left.* Hepatic vein branch in dog liver, with toothlike projections composed of spiral muscles. H&E ($\times 85$). *Lower right.* Contracted hepatic vein branch in dog liver. The surrounding central lymphatic vessels are open. H&E ($\times 85$).

in man, in contrast to that in the dog, and the blood of the hepatic vein enters almost directly into the right atrium. This relation facilitates the catheterization of the hepatic veins.

Hepatic Vein Sphincters in Various Animals. The histology of the hepatic veins varies in different animals, especially in the sphincter arrangements. Outlet sphincters in venules have been observed by vital microscopy [1808]. The cat is similar to man in this respect [2625], while the

dog and aquatic mammals, such as the seal, have a muscular throttle mechanism in the hepatic veins (Fig. 53, lower left and right). In these animals, spiral or longitudinal muscle columns start within the central vein and extend into the collecting veins. These muscle bundles project like the teeth of a saw into the lumen, and when contracted they almost completely obstruct the lumen. Other animals, such as the guinea pig, have circular sphincters at the hepatic vein ostiums [3170]. Various



FIG. 54 Interdigitation of portal (light) and hepatic (dark) venous branches. (From original of Fig. 19, Elias, H., and Petty, D.: *Am.J.Anat.* 90:89, 1952.)

combinations of the intrahepatic or extrahepatic sphincter mechanisms can be noted in other animals [3341].

STRUCTURAL PRINCIPLE OF THE VASCULAR TREE

The principle of the lobule as the structural unit of the liver involves a complete separation of the branches of the portal and hepatic veins. The branches are normally arranged in an interdigitated fashion, in that lobular parenchyma is interposed in all planes of the liver between the terminal branches of the portal vein and the initial branches of the hepatic vein (Fig. 54). The distance between the branches is the radius of the lobule. On the basis of reconstruction experiments, Pfuhl assumed this radius to be equal in all planes of all lobules throughout the liver, the distance between the terminal ends of both venous systems being constant [2582]. Variations in this distance were said to cause blood to flow through the sinusoids of shorter length, thus depriving the longer ones of adequate blood. This statement is correct in principle as far as extreme variations in pathologic processes are concerned. It does not take into account variations in venous pressures and contractions of the sinusoidal walls and

sphincters which compensate for the differences in the lengths of the sinusoids shown by reconstruction studies [907].

Intrahepatic Vascular Anastomoses and Mixing of Blood. Vital microscopic observations and injection preparations indicate that some parts of the lobule contain mixed blood and others pure arterial or venous blood. They offer conflicting evidence as to whether portal venous and hepatic arterial blood mix before entering the lobule or only within the lobule.

VITAL MICROSCOPIC OBSERVATIONS. Vital microscopy of the mammalian and the frog liver demonstrated that branches of the portal vein and the hepatic artery on the periphery of the lobule divide into fine ramifications which extend throughout the lobule independently of one another [1808, 3463]. In the periphery of the lobule the flow in the arterial branch is faster than in the portal branch, while in the central region where the sinusoids converge toward the central vein, this difference disappears. Thus parts of the lobule receive only arterial blood, and other parts only portal blood. The sinusoids are constricted at their origins from the portal vein or the hepatic artery and at their entrance into the hepatic vein, with ampulla formation at their terminal position. In addition to the mixing of portal and arterial blood

in the sinusoids, larger arterial branches may anastomose with portal veins through small direct communicating branches or by emptying entirely into the portal vein in the interlobular region or at the periphery of the lobule. Communications between hepatic artery and hepatic vein branches are seen only in amphibian livers [3463].

INJECTION PREPARATIONS. In injection preparations, direct anastomoses between interlobular portal veins and small branches of the hepatic artery could not be demonstrated in the normal liver; the only communications found occurred through the sinusoids and the capillary plexus in the portal canal [907]. The communications seen during vital microscopy apparently take place

through straight dilated arterioles, capillaries, and venules which simulate precapillary anastomoses [907]. The communication thus occurs between the radicular branches of the hepatic artery, the peribiliary plexus, and the internal radicles of the portal vein (Figs. 45 and 49).

ANASTOMOSES BETWEEN PORTAL AND HEPATIC VEIN BRANCHES. Direct anastomoses between portal vein and hepatic vein branches, bypassing the lobular parenchyma, have also been claimed and are supposedly open in the absence of digestion or activity [1996]. Functionally, in rabbits, intrahepatic anastomoses accommodating glass spheres up to $180\ \mu$ in diameter can be demonstrated without proof of their exact site [2671].

18

FUNCTION OF HEPATIC BLOOD VESSELS

The hepatic circulation plays an important role in hepatic function, in view of the intimate contact necessary between the hepatic parenchyma and the blood in the sinusoids. Hepatic function is controlled not only by the function of the parenchyma but also by the amount and rate of flow of the blood passing through the liver. The unusual means of blood supply, consisting of two afferent channels, the hepatic artery and the portal vein, necessitates mixing of venous and arterial blood. The contribution of each afferent channel, as well as the mechanism regulating the mixture and distribution of the blood flow, is discussed separately, following a description of the measurement of the entire blood flow and the pharmacodynamic influences, without consideration of the contribution of either vessel.

Total Hepatic Blood Flow

METHODS OF MEASUREMENT. The total hepatic blood flow can be measured by direct methods in animal or cadaver liver and by recently introduced indirect methods in living persons. Various experimental procedures have been described for direct measurements [1175], some using the thermistor-muhr [1285, 1291]; the necessary surgical procedures, however, alter the results significantly. Postmortal injection studies are also inaccurate. Venous catheterization makes it possible to obtain blood from the hepatic vein branches in the living person [373], permitting the determination of the blood flow on the basis of the Fick principle. According to this principle, the amount of a substance removed per minute from the blood divided by the amount removed from each milliliter of blood passing through the organ equals the rate of blood flow. The use of this principle, often applied for calculation of the blood flow, requires

knowledge of (1) the concentration of a substance in the blood flowing into an organ; (2) its concentration in blood leaving the organ; (3) the total amount removed from the blood by the organ per unit of time. The first factor can be approximated by determination of peripheral blood levels. The second factor is determined in the hepatic venous blood. The third factor is the amount which has to be injected to maintain a constant blood level, the amount presumably removed from the blood by the liver. The substance mainly used has been Bromsulphalein [373]. This application of the Fick principle contains several sources of error. These are:

1. The peripheral blood Bromsulphalein concentration may not necessarily be the concentration entering the liver. This source of error is probably of minor significance.
2. The blood obtained from one hepatic vein may not be representative of all.
3. Bromsulphalein may be extracted by other organs [2999] and may be returned to the blood from the intestine [2064]. If the Bromsulphalein level is kept between 1 and 2 mg per 100 ml, this source of error seems to be rather insignificant [3043].
4. Some blood from the splanchnic area may bypass the liver, either by way of collaterals or, less likely, by way of lymphatic vessels. In the absence of large collaterals, this amount is probably insignificant.

For these reasons the term "estimated hepatic blood flow" [373] or "estimated splanchnic blood flow" [3043] is used. The validity of the basic principle is supported by observations based on the estimation of urea production [2397]. The average blood flow is approximately 1,500 ml per minute per 1.73 sq m body surface. Transfusion

studies on post-mortem specimens result in a higher figure, almost 2,500 ml per minute, as a summation of total hepatic artery and portal vein flow [805]. Results in animals, particularly dogs, based primarily on stromuhr and catheterization methods, are similar to those in man [515, 684, 1285]. This means that approximately one-fourth of the basal cardiac output passes through the splanchnic area. The amount varies less than the blood flow through other organs studied. Variations of the flow in the different hepatic vein branches are relatively small [370].

PHYSIOLOGIC INFLUENCES. Exercise and an upright position decrease the hepatic blood flow; eating has no effect upon it [370, 696]. Pregnancy does not influence hepatic blood flow, despite an increase in the total blood volume [2378]. In animal experiments, electrical stimulation of the vagus nerves does not influence blood flow, whereas hepatic plexus stimulation does [3462]. Sympathetic nerve tone, together with the arterial blood pressure, is an important factor in controlling the hepatic blood flow [3601]. Blood pressure elevation increases the flow, while reduction decreases it [1175] (Table 7).

Table 7 Physiologic Changes in Hepatic Blood Flow

<i>Flow Decreased</i> (<i>Splanchnic</i> <i>Vasoconstriction</i>)	<i>Flow Increased</i> (<i>Splanchnic</i> <i>Vasodilatation</i>)
Vertical posture	Fever
Exercise	Killed bacteria
Fainting	Alcohol
Norepinephrine	Epinephrine
General anesthesia	Sympathectomy

Courtesy of Dr. Sheldon S. Waldstein.

PHARMACOLOGIC INFLUENCES. During pyrogenic reactions the hepatic blood flow rises, whereas during anesthesia [3013] it drops. Both phenomena are expressions of parallel alterations in the peripheral blood flow [371]. Intravenous infusions of saline, glucose, or sucrose increase hepatic blood flow in both the portal vein and hepatic artery [3462]. Epinephrine increases the hepatic blood flow [3041], although rapid infusion of intravenous or intraportal epinephrine decreases it [1175]. Since the blood pressure does not significantly rise, the total hepatic or splanchnic resistance is probably reduced. Norepinephrine raises the blood pressure without significantly influenc-

ing the estimated hepatic or splanchnic blood flow. Epinephrine and norepinephrine act upon the splanchnic arterioles rather than upon the hepatic sinusoids [2652, 3104]. In perfusion experiments, the response to epinephrine is altered if the liver has been subjected to a period of anoxia [2175]. Bile acids, cinchophen, acetylcholine, and hexamethonium increase the hepatic arterial blood flow [1175, 1291]. Alcohol has no effect on hepatic blood flow [3105].

VARIATIONS IN SINUSOIDAL BLOOD FLOW THROUGHOUT THE LOBULE. Vital microscopic observations in rats and frogs showed an intermittent rhythmicity of sinusoidal contractions in different territories throughout the liver and even within the same lobule [1808, 3463]. In some lobules almost all sinusoids contain motionless red cells, some are distended with cells, and others are almost empty, indicating contractibility. The distribution of inactive lobules or parts of lobules changes rapidly in an irregular fashion. Under basal conditions 75 per cent of the sinusoids are inactive. One part of a lobule may remain inactive while neighboring territories undergo rapid shifts, although even in active sinusoids the rate of flow varies considerably. A blood-storing function is indicated by the alternating periods of increased and decreased flow [1285]. The extent to which all these observations apply to man is not established.

ABNORMALITIES. In cirrhosis, the hepatic blood flow is low or normal regardless of the type of

Table 8 Variations of the Estimated Hepatic Blood Flow (EHBF), Bromsulfalein Extraction (Ext. BSP), Arteriovenous (AV) Oxygen Difference (in the Hepatic Vein), and Splanchnic Oxygen Consumption

<i>Condition</i>	<i>EHBF</i>	<i>Ext. BSP</i>	<i>AV oxygen difference</i>	<i>Splanchnic oxygen consumption</i>
Normal.....	1,500 cc./in.	50%	4 vol %	60 cc./min.
Cirrhosis.....	↓	↓	↑	N
Cirrhosis after portocaval shunt.....	↓	↑	↑	N
Fatty liver.....	N	↓	↑	↑
Hypertension.....	N	N	N	N
Heart failure.....	↓	↓	↑	N
Hyperthyroidism.....	N	↓	↑	↑
Pregnancy (including toxemia).....	N	N		
Diabetes mellitus.....	N		N	N

Courtesy of Dr. Sheldon S. Waldstein.

cirrhosis [374, 2397]. After splenorenal or portocaval shunt operations it is even lower. In cardiac failure, hepatic blood flow is moderately reduced [2399]. In hyperthyroidism, it is not increased, in spite of the increased cardiac output [2398]. Experimental ischemia [515] or hemorrhage [1447] reduces the hepatic blood flow. The reduction of the estimated hepatic blood flow in cirrhosis is associated with an increased arteriovenous oxygen difference (Table 8), so that the total splanchnic oxygen consumption is not altered. In fatty livers, on the other hand, a normal blood flow is associated with an increased arteriovenous oxygen difference and increased splanchnic oxygen consumption. Bromsulphalein extraction by the liver is reduced in cirrhosis and also in fatty liver, despite the normal blood flow, suggesting that parenchymal hepatic damage may overshadow circulatory insufficiency under these circumstances [1733].

Relation of Portal Vein to Hepatic Artery Blood Flow. Anatomically, the lumen of the hepatic artery is considerably smaller than that of the portal vein. Since the blood in the hepatic artery is under considerably higher pressure than the blood in the portal vein, the contribution of each vessel to the total hepatic blood flow and to the total oxygen supply is not easily evaluated. Actually, great variations are found in the figures reported. The portal vein provides, at least under normal conditions, the bulk of the blood flowing to the liver, with 10 to 35 per cent of its blood coming from the spleen. Earlier figures indicated that one-fourth to one-third of the flow through the liver comes from the hepatic artery and the rest from the portal vein. Examinations on unanesthetized dogs show that about 20 per cent of the blood comes from the hepatic artery and 80 per cent from the portal vein [1285], although under some circumstances as much as 90 per cent of the blood may come from either vessel [3139]. Indirect measurements in man also indicate great variations [2397]. Species differences are probably important [2158]. In general, a reciprocal relation exists between hepatic artery and portal vein [1285], in that experimental narrowing of the portal vein or heating of the animals decreases the portal vein flow with a simultaneous increase in hepatic vein flow. The amount of oxygen provided to the liver by the artery and vein also varies, with 60 to 80 per cent of the oxygen coming from the portal blood [2158]. However, some

assume that 60 per cent may be provided by the hepatic artery.

PORTAL VEIN

The function of the portal vein is the drainage of the splanchnic system into the liver. The liver does not require portal vein blood for its basal functions but seems to need it for regeneration.

STREAMLINES OF FLOW. Blood from the splenic, coronary, and inferior mesenteric veins goes to the left lobe of the liver, while blood from the superior mesenteric vein goes to the right lobe. This means that blood from the spleen, body and tail of the pancreas, stomach, first portion of the duodenum, and the left half of the colon usually is drained into the left lobe, while blood from the small intestine and that from the right half of the colon goes to the right lobe. This principle has been proved with the aid of dye injections in transilluminated livers and with radioactive phosphorus either in solution [1338] or incorporated into small wax spherules injected into the contributory vessels of experimental animals [1338, 1497]. The extent to which the streamlines of flow occur in man is unknown. No differences are noted in visualization of the vessels during splenoportography, whether the contrast medium is injected into the spleen or into the mesenteric veins. Also, injection of cadaver livers failed to confirm the principle of streamlines.

These streamlines of portal flow have been said to determine in part the localization of necrotizing processes, abscesses, or tumor metastases. They do not result in chemical differences between the lobes of the liver, since the mixing of blood is complete after the first passage through the liver, as demonstrated by the equal distribution of bacteria injected into the jugular vein [1338]. The greater incidence of necrosis induced by viruses or deficiency in the left lobe is the result of better protection of the right lobe afforded by foods, particularly amino acids, absorbed from the intestine [1497]. In intrauterine life, the left lobe receives more oxygen from the umbilical vein, and therefore anoxia produces more severe changes in the right lobe [1304]. After birth, the left lobe, accustomed to a high oxygen content, may show signs of necrosis. The middle portion of the liver, the areas of the caudate and quadrate lobes and the areas superior and posterior to them, receives mixed blood

The blood flow through the periphery of the liver varies in portal venographic studies, regardless of the lobe. Under certain experimental conditions the blood was found short-circuited through central intrahepatic pathways, while the periphery was not perfused [2670].

PORTAL VEIN PRESSURE. Determination of the pressure in the portal vein has not yielded uniform results because of the difficulties inherent in any measurement of venous pressure, particularly under anesthesia. In unanesthetized dogs pressures of 3 to 11 cm water with an average of 6.0 cm were found with a portocaval gradient of 5 to 9 cm. This finding agrees with other measurements of 7 to 11 cm in normal dogs [3439] and similar figures in cats [2158]. In man, great variations have been reported, depending upon the technique used and the condition of the patient [298, 2840]. The range is similar to that found in animals, and the pressure is always greater than in the systemic veins, especially the inferior vena cava. Exercise does not influence portal pressure,

but ingestion of food increases it [1517]. By simultaneous occlusion of hepatic and portal vein tributaries, the hepatic sinusoidal pressure can be estimated as the mid-point between the pressures necessary for occlusion of each vessel. The average value of this sinusoidal pressure is 11.5 cm water in dogs [1099]. Various indirect methods of measuring portal pressure have been used, primarily to evaluate portal hypertension (see Portal Hypertension, Chap. 29). These include pressures in collaterals such as hemorrhoidal or abdominal veins, intrasplenic pressure, and pressures in hepatic vein radicles occluded by a catheter (wedge pressure). Portal circulation time has been measured by the instillation of ether into the rectum [2435, 3466]. This is increased in portal hypertension and in many other conditions, so that it is of little diagnostic value.

PORTAL VEIN OXYGEN. The oxygen saturation of the portal blood varies between 10 and 68 per cent and is directly dependent upon the systemic blood pressures [2158]. In the rabbit, where the

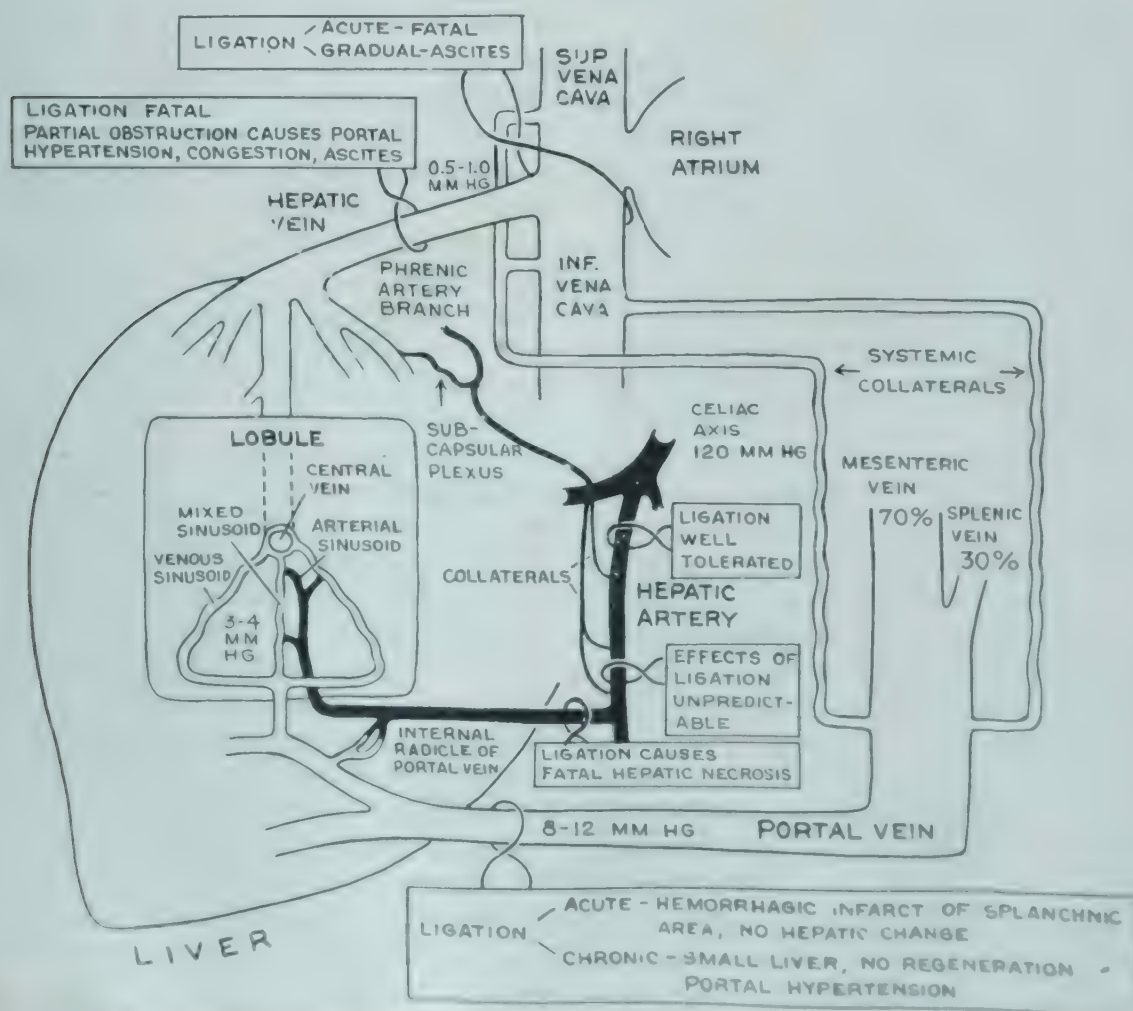


FIG. 55 Pressures and effects of ligations in the blood vessels of the liver.

portal blood supplies little oxygen to the liver, the average portal oxygen saturation is 27 per cent. In general, the oxygen content of the portal vein blood is higher than that of systemic veins and only slightly lower than that of arterial blood [375]. In the dog, the oxygen content is higher than in man.

Interference with Portal Blood Flow

The rate of flow in the portal vein varies between 145 and 505 ml per minute in the dog [1285]. Various factors can interfere with portal blood flow. In each, the effect upon the liver has to be differentiated from the effect upon the other splanchnic organs. Experimental results assist in the understanding of the clinical and pathologic findings in man (Fig. 55).

Sudden Portal Vein Obstruction. IN ANIMALS.

Sudden obstruction of the portal vein in dogs or cats leads to death in shock within 24 hours, owing mainly to stasis of blood in the splanchnic system [565]. In monkeys sudden ligation does not necessarily lead to death, probably because the elevation of the portal pressure opens sufficient collaterals to permit discharge of the blood.

IN MAN. Until recently sudden complete obstruction of the portal vein by a thrombus forming in the portal vein or extending into it from the splenic or mesenteric veins was thought to lead rapidly to an abdominal catastrophe with ascites, hemorrhagic infarction of the intestine, and death (see Portal Vein Thrombosis, under Portal Hypertension, Chap. 29). At autopsy no specific lesions are found in the liver. Because occlusion of the portal vein during surgery failed to produce the findings described (although the patients died), and because of the experiences recorded in monkeys, a sudden and complete ligation of the portal vein in man was tried [565]. It did not produce the recognized picture of fatal portal vein thrombosis and did not alter hepatic function. The blood pressure dropped, and the intestine was engorged only temporarily. Apparently surgical ligation of the portal vein is feasible if it is required for surgery of the pancreas or duodenum. These results indicate that the catastrophic symptoms in man are produced by simultaneous thrombosis of the mesenteric and splenic veins which is usually associated with spontaneous portal vein thrombosis. Thrombosis of the mesenteric branches alone leads to almost identical changes [2457].

Thrombosis of branches of the portal vein in the liver occurs spontaneously in the preterminal

period owing to circulatory insufficiency or to tumor metastases. A red, atrophic infarct (Zahn infarct) results from stasis of blood in the draining hepatic vein because of a lack of portal pressure after obstruction associated with a lowered hepatic arterial pressure. Grossly, the wedge-shaped hyperemic zone is sharply delineated from the surrounding parenchyma (Fig. 56, upper and lower). Histologically, severe acute passive congestion is noted, with atrophy of the cells in the centrolobular zone, along with the development of bridges of congested hepatic tissue connecting adjacent central areas. In man, oxygen want owing to portal vein thrombosis, without complicating arterial hypotension or obstruction, does not produce an anemic infarct except in the prenatal period [1684].

Gradual Compression of the Portal Vein. IN

ANIMALS. Gradual interruption of the portal circulation is well tolerated in rabbits, cats, and dogs [565, 1497, 2435], owing to the development of extrahepatic collaterals which effectively bypass the obstruction. These collaterals prevent extrahepatic changes ordinarily resulting from obstruction of the main stem of the portal vein in these animals. Experimentally, ascites does not result from gradual ligation of the portal vein [1517] except if associated with serum protein depletion by plasmapheresis. Portal obstruction deprives the liver of some of its oxygen and of nutrients from the intestine, and it suppresses regeneration (see Circulation, under Factors Influencing Regeneration, Chap. 13). In rabbits, where little oxygen comes from the portal vein, ligation of the portal vein leads to atrophy without necrosis. In cats, where the liver obtains much of its oxygen from the portal blood, ligation leads to severe fatty changes with necrosis which are only secondarily followed by atrophy [1497, 2435]. Hepatic fibrosis after obstruction of the portal vein is not entirely the result of oxygen want [1497]. It is probably caused by nutritional deficiencies, since oxygen supplied by the hepatic artery should be able to maintain the viability of a larger portion of the parenchymal tissue than is actually found.

IN MAN. Slow interruption of portal blood flow by a gradually developing thrombus leads to extensive collaterals which drain blood through the hepatoduodenal ligament from below the obstruction either to the liver (hepatopetal collaterals) or to the systemic circulation (hepatofugal collaterals). The thrombus itself may become recanalized, as in cavernomatous transformation (see In-

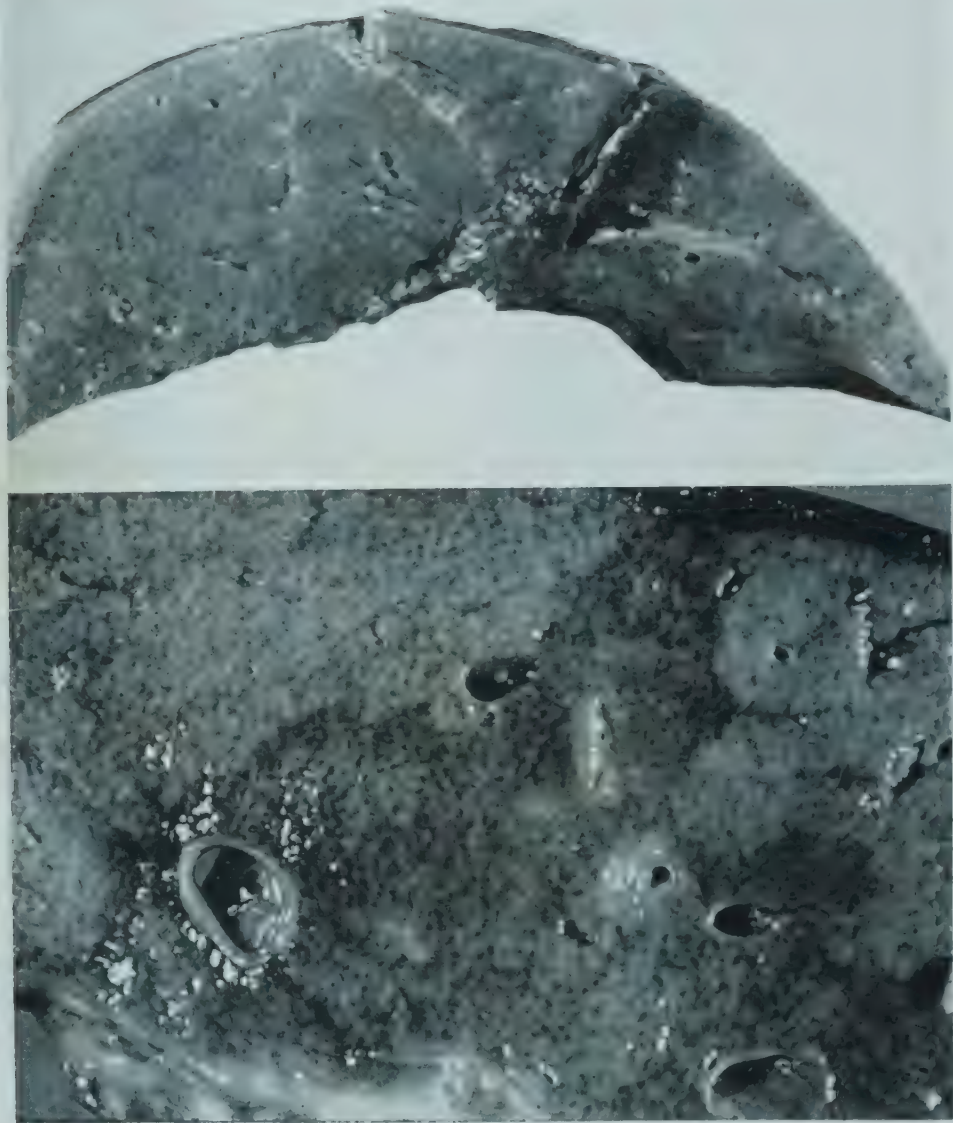


FIG. 56 Cross section (*upper*) and close-up (*lower*) of Zahn infarct caused by terminal thrombosis of portal vein branches, represented by the darker area.

frahepatic Portal Hypertension, Chap. 29). Under these conditions, the liver shows only slight fibrosis [1497].

Eck Fistula. A classic method of altering portal vein circulation is the creation of an Eck fistula, first done in 1877 and consisting of a side-to-side anastomosis of the portal vein and vena cava, with ligation of the portal vein between the anastomosis and the liver [566]. Animals with such anastomoses show evidences of hepatic insufficiency, such as intoxication with neurologic symptoms terminating in coma after ingesting meat [638, 1088, 2202]. In the animal with an Eck fistula, the liver is slightly atrophic. The production of plasma proteins, hemoglobin, and bile pigment is reduced and serum-lipid levels are low [3574, 3628]. Oral administration of water readily pro-

duces hemodilution. Female animals do not menstruate or conceive.

In man, the principle of the Eck fistula in the form of portocaval shunts has been applied for portal decompression (see Venous Shunt Operations, under Portal Hypertension, Chap. 29).

REVERSED ECK FISTULA. Ligation of the vena cava instead of the portal vein above the portocaval anastomosis diverts all blood from the hind part of the body to the liver, which enlarges and shows vascular engorgement. Since abdominal collaterals form, this operation is used prior to experimental hepatectomy [3462].

Arterialization of Portal Vein. Repeated attempts have been made to anastomose branches of the hepatic artery with the portal vein [565]. This leads to increased oxygen saturation of portal

blood [2926], but in most instances severe hepatic congestion is produced. Further technical improvements are required before the procedure can be applied successfully, experimentally or clinically.

HEPATIC ARTERY

The chief function of the hepatic artery is to supply oxygen to the parenchymal cells. The hepatic artery behaves like any systemic artery as far as its pressure and oxygen saturation are concerned. The flow through it ranges from 44.6 to 163 ml per minute in dogs [1285]. Reactive hyperemia following temporary interruption of the blood flow is not seen in the liver as in other organs [2158]. Reduction of portal blood flow [1285] or intravenous administration of sodium dehydrocholate or cinchophen [1291] increases the hepatic arterial blood flow.

Interruption of Hepatic Artery Blood Flow. IN ANIMALS. The contribution of the hepatic artery to hepatic blood flow varies greatly, depending upon the portal blood flow (Fig. 55). After constriction of the hepatic artery, the oxygen content in the hepatic vein falls, sometimes to less than 1.0 per cent. In animals with hemorrhagic shock, the arteriohepatic venous oxygen difference increases [1369]. The liver requires arterial blood, although the experimental evidence derived from ligation of the artery is complicated by species differences. In dogs and cats the consequences of ligation of the hepatic artery depend upon the site of ligation [565, 2220]. If it is performed near the point of origin of the hepatic artery from the aorta, the animal will survive. The closer the ligation is to the liver, the greater the mortality rate, because the extensive extrahepatic collaterals, especially from phrenic arteries, are no longer able to supply arterial blood to the liver. If ligation is complete, which requires careful operative technique in view of the many collaterals [2220], rapid necrosis of the liver and gallbladder develops, and death results [2653, 2654]. In the dog the oxygen is primarily required to inhibit growth of anaerobic bacteria sensitive to penicillin. Penicillin treatment immediately following complete, otherwise fatal, ligation of the artery preserves the dog's life. Discontinuation of the penicillin treatment after several days is well tolerated, probably because collaterals have formed. Dogs tolerate gradual dearterialization even if the portal vein is subsequently excised and little blood reaches the liver,

which has slowly become adapted to a reduced blood flow [2654]. Incomplete obstruction causes central hepatic necrosis [2220]. In monkeys, extirpation of the entire hepatic artery with all its branches is well tolerated, although small scattered infarcts with atrophy of the gallbladder develop.

IN MAN. Oxygen plays a major role in the maintenance of the human liver; as indicated by the hepatic degeneration in the right lobe in anoxic infants, in whom the left lobe is protected by umbilical vein blood [1304] (see Streamlines of Flow, under Portal Vein, earlier in this chapter). Effective interference with the arterial blood supply to the liver produces anemic infarcts (Fig. 57, top). Incomplete interruption of the hepatic artery flow leads to central zonal necrosis [1497, 2220], while complete interruption leads to massive or anoxic necrosis (Fig. 57, middle) (see Anoxic Necrosis, under Necrosis, Chap. 22).

LIGATION OF HEPATIC ARTERY. Accidental ligation or interruption of hepatic artery flow produces divergent and unpredictable results, depending on the existing collaterals [2653]. Ligation of the common hepatic artery, therefore, is usually compatible with life in man. Ligation after the origin of the gastroduodenal artery is usually fatal, because the collateral circulation usually does not suffice (Fig. 57, bottom). In view of the variations of the hepatic arterial branches [565, 2220, 2284], the results of ligation at the same site may differ from patient to patient. In cirrhosis the dangers of hepatic artery ligation appear reduced, because the relation between the hepatic artery and portal vein contributions is altered [565]. Since suppression of anaerobic bacteria is not a problem after hepatic artery ligation, the use of antibiotics is not particularly necessary.

ANEMIC INFARCTS. The anemic infarct is the result of interruption of the entire blood supply to an area. Clinically, anemic infarction causes severe toxic manifestations with fever and shock. Anemic infarcts of the liver may occur in any lobe. They range in color from gray brown to bright yellow and are always lighter than the surrounding tissue. They vary in size from barely visible lesions to half the liver. They are sharply circumscribed and are prominent on the cut surface (Fig. 57, top). Infarcts were found 54 times in 18,230 autopsies [3657]. Histologically, in earlier stages, the brightly eosinophilic epithelial cells, without nuclear staining, remain in place, and a zone of intact cells is preserved around the portal tracts.



FIG. 57 *Top.* Anemic infarct of liver in patient with peritonitis without grossly demonstrable obstruction of hepatic artery or portal vein. *Center.* Massive ischemic necrosis with remnants of viable hepatic cells around some portal tracts in the vicinity of traumatic injury to the liver. H&E ($\times 65$). *Bottom.* Ischemic necrosis of the left lobe of the liver after hepatic artery ligation.

Some of the mesenchymal elements, especially in the portal and central canals, are also preserved, since they are more resistant to anoxia. Leukocytic infiltration within the area is not conspicuous, but a demarcation zone of segmented leukocytes is common (Fig. 92, lower right). Anemic infarcts may be the result of arterial obstruction. Arterial emboli rarely reach the liver because of the complex course of the hepatic artery outside the liver. Hepatic artery occlusion occurs following embolism of the aorta in children [2347]. Such emboli result more commonly from subacute bacterial endocarditis than from mural thrombi in the heart or from paradoxical embolisms [2068]. Thrombosis caused by surrounding infections or carcinoma is rare. It is more often produced by aneurysms of the hepatic artery (see Aneurysms of Hepatic Arteries, under Diseases of Hepatic Artery, Chap. 56). The most frequent cause of obstruction is polyarteritis nodosa (see Polyarteritis Nodosa, under Diseases of Hepatic Artery, Chap. 56). Another common cause of hepatic infarction is trauma. The development of hepatic infarcts is unpredictable, and in many instances no occlusion of a hepatic artery branch is demonstrable. In some instances portal vein occlusion complicated by local or systemic anoxia may be responsible, although in one-third of cases no vascular occlusions of any sort are found [3657].

In conclusion, some hepatic arterial blood appears to be essential to provide sufficient oxygen for metabolic functions, since the portal vein oxygen tension is not high enough as it is in dogs. The unpredictable contribution of oxygen by collateral arteries results in unforeseen sequelae of ligations of the hepatic artery in a critical area between the gastroduodenal artery and the porta hepatis.

HEPATIC VEIN

The blood flow through the hepatic vein is, in effect, identical with the total hepatic blood flow. The blood pressure in the large hepatic veins is essentially the same as the pressure in the vena cava, except for the effects of the sphincters within the liver and at the entrance of the hepatic veins into the vena cava. The oxygen saturation varies in different species. In the cat, for instance, in which the portal vein supplies much of the oxygen to the liver, the hepatic venous oxygen saturation is low [2158]. In man, it is considerably higher and approaches or exceeds the oxygen saturation

of systemic venous blood (60 to 85 per cent) [371].

INTERFERENCE WITH HEPATIC VEIN FLOW. Complete experimental obstruction of the hepatic veins is tolerated only in the presence of a portocaval shunt [692]. If the hepatic vein of the monkey, which tolerates portal vein occlusion well, is obstructed without interfering with the flow in the inferior vena cava, the animal dies rapidly, owing to engorgement of the splanchnic venous bed (Fig. 55). Apparently the portal collaterals are unable to remove the added hepatic arterial inflow [565]. Complete obstruction of the supradiaphragmatic portion of the inferior vena cava in the dog rapidly causes death, with a fall in arterial pressure and an increase in lymph flow [340].

Partial obstruction of the hepatic veins leads to changes similar to those produced by very severe passive congestion. Partial obstruction of the inferior vena cava in the dog leads to ascites formation, owing not only to stasis but also to excessive loss of hepatic lymph into the peritoneum [3439]. Partial obstruction or narrowing of the hepatic veins produces ascites. Subacute obstruction of the hepatic veins in man is caused by thrombosis, tumors within the liver, or abscesses within or around the liver. This obstruction is accompanied by severe pain, nausea, rapidly increasing hepatosplenomegaly, and deterioration of hepatic function. In the more chronic forms, resulting mainly from endophlebitis (Chiari syndrome), pain and gradual enlargement are combined with evidence of portal hypertension [1722] (see Portal Hypertension, Chap. 29). Centrilobular necrosis is the predominant finding in the acute stage, while fibrosis is noted in the chronic stage. The obstruction by regenerative nodules within the liver that occurs in cirrhosis is discussed under portal hypertension (see Portal Hypertension, Chap. 29).

DIFFERENCES IN COMPOSITION OF BLOOD IN THE VARIOUS VESSELS

The portal vein blood differs from the hepatic arterial blood primarily because of admixture of substances absorbed from the intestinal tract. The difference in oxygen saturation between these two vessels is considerably smaller than the systemic arteriovenous (A-V) oxygen difference, particularly in cirrhosis [2397, 3439]. The difference between portal and hepatic venous blood has been repeatedly measured to determine the uptake or

release of substances from the liver in animals. The experimental conditions, however, do not necessarily reflect the differences in the unanesthetized state. Recently, hepatic blood obtained by catheterization has been compared with peripheral arterial blood, considered identical to hepatic artery blood. The A-V oxygen difference thus determined indicates that the liver extracts a greater amount of oxygen than the other splanchnic organs [3439]. The A-V difference under normal circumstances with normal hepatic venous oxygen saturation is relatively low, 2.5 to 5.0 vol per cent, in comparison with that of the systemic circulation, despite the metabolic function of the organ [370]. This is explained by the rapid flow of the blood through the liver, as well as by the large volume of blood. Also, under normal circumstances, the blood flow is not retarded at any point within the liver, and therefore no pools of stagnating blood exist, as they do in the spleen. Under abnormal circumstances, as in cirrhosis, the A-V oxygen difference is increased parallel with a reduction of the oxygen saturation [370, 3439], possibly owing to slowing of the blood flow. Slowing of the blood flow increases the oxygen extraction per unit of blood. The oxygen consumption of hepatic tissue seems to be fairly constant, in contrast to that of the kidney [370]. From the blood flow and the oxygen concentration, the splanchnic oxygen consumption can be determined. This rises after administration of epinephrine parallel with certain metabolic functions of the liver, such as hepatic lactic acid uptake [3041]. Epinephrine also increases the hepatic glucose output, as determined from splanchnic blood flow and the arterial or capillary glucose concentration [3041], indicating glucose release by the liver.

Partial Obstruction of More than One Vessel. The surprising tolerance of various species of animals, probably including man, to complete obstruction of the portal vein and almost complete occlusion of the hepatic arterial flow is explained by the mutual substitution of hepatic arterial and portal venous blood. In the monkey, simultaneous ligation of the hepatic artery and the portal vein is fatal, with the development of massive hepatic necrosis [565]. Ligation of the hepatic artery followed by portal vein occlusion is more readily tolerated than the reverse procedure, since the hepatic arterial collaterals have time to develop [2654]. Gradual interference with blood flow in both vessels in dogs protected with penicillin leads

to hepatic coma [2710] (see Hepatic Coma, Chap. 23).

REGULATION OF HEPATIC BLOOD FLOW

The blood flow through the liver depends to a great degree on the amount of blood offered to the liver from the portal tributaries, i.e., on the activity of the splanchnic vessels and the regulatory mechanisms governing them. The variations in hepatic blood flow which result from shock and other alterations of the blood pressure are attributable primarily to alterations in the splanchnic flow. In this sense, the amount of blood passing through the main portal vein is independent of the liver itself. To compensate in part for alterations in portal flow, changes in the hepatic arterial flow occur. Morphologic as well as functional studies suggest a rather intricate system of sphincter or throttle mechanisms, mainly in the arteries and veins, to regulate the blood flow within the liver. This specific regulatory system determines (1) the capillarization of the sinusoids and whether plasma or whole blood passes through them; (2) the length of time a given blood column is exposed to the metabolic influence of the hepatic cells; (3) the water-storing ability of the liver. Finally, it regulates the blood pressure where portal vein and hepatic artery blood mix within the liver.

SPHINCTERS IN THE HEPATIC ARTERY SYSTEM. The sphincter system of the hepatic artery (see Intrahepatic Portion, The Hepatic Artery, Chap. 17) serves two purposes. The first is to reduce the arterial blood pressure prior to the confluence of arterial and venous blood. This may also be facilitated by the tortuosity of the arteries. Secondly, it serves to regulate the entrance of the blood into the lobule at different levels.

SPHINCTERS IN THE PORTAL SYSTEM. The sphincters in the portal inlet venules (see Venules, The Portal Vein, Chap. 17) regulate the degree and type of capillarization of the sinusoids. Little is known about physiologic and pharmacologic effects upon this mechanism.

HEPATIC VEIN SPHINCTERS. The hepatic venous branches contain the most conspicuous sphincter mechanism, with many species differences (Fig. 53). Observations on experimental animals, such as the dog, therefore, are not necessarily applicable to man. Sphincters in the larger vessels exist in all animals, although in man they normally contract very slightly, as evidenced by the ease with

high catheters enter the liver from the vena cava. In man relatively thin central veins pierce through thick-walled, firm, sublobular, and larger hepatic veins, and contraction of the larger veins occludes the smaller ones, which may show a sinuslike dilatation before entering into a larger vein. Little is established about the functional effect of the sphincters in man.

Sphincters in the Dog. The intrahepatic throttle mechanism is most conspicuous in the dog and is best studied when constriction has been effected by peptone, histamine, anaphylactic shock, digitoxin, hydatid cyst fluid, or ascaris extract with subsequent enlargement of the liver, even in vitro [2248, 3323]. Digitoxin presumably increases resistance to the hepatic vein, resulting in increased storage of blood in the liver [1701]. Atropine and pinephrine, in small doses, open the sphincters and reduce the size of the liver, whereas pilocarpine, eserine, and Mecholyl cause the sphincters to contract and the liver to enlarge [780]. The contraction of these sphincters in shock is probably the main cause of the functional and structural damage. These sphincters aid in the distribution of water, in that fluid may be expressed into the lymphatic vessels, bypassing the inferior vena cava [2248], and in shock the lymphatic vessels appear widely dilated [2625]. In addition, by closing the throttle mechanism, the liver retains blood and thus increases its reservoir function for blood. This is important, since the splanchnic area is a major blood depot and may contain 55 per cent of the total blood volume. The diversion of a large amount of blood owing to contraction of the sphincters may, in itself, result in shock. The reason for the elaborate sphincter mechanism in the dog is not known.

Sphincters in Other Animals. A conspicuous sphincter mechanism in aquatic mammals has been connected with submersion. Such a mechanism has been assumed to exist in other animals on the basis of functional effects without an obvious muscular throttle [780, 3122, 3323]. In cats, for instance, this mechanism has been associated with the contraction of the small endothelial sluice channels which pass through the thick walls of the sublobular veins. Closing of these sluice channels forces blood to go through the central vein, resulting in a sphincterlike effect. These channels respond to drugs in the same way as do the sphincters in dogs [780].

INTERPLAY OF SPHINCTERS. The blood flow through the liver depends upon the interplay of

the sphincters, which determines not only which column of blood is in contact with what part of the hepatic plates, but also for how long. The nervous influence upon the blood flow depends partly upon these sphincters, and the liver shrinks as a result of splanchnic or hepatic nerve stimulation [3462]. The normal regulatory mechanism

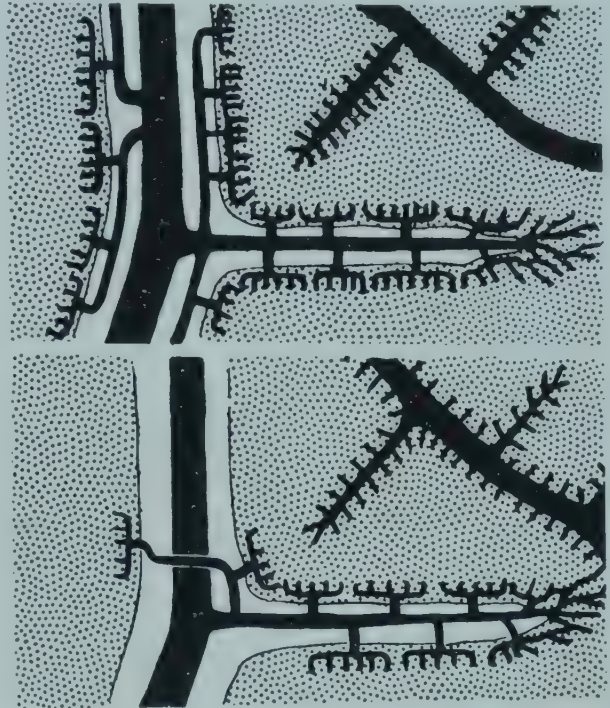


FIG. 58 Schematic drawing showing distribution of portal and hepatic veins. *Upper.* Man. The parenchyma around large portal tracts receives blood directly from small distributing portal veins accompanying the large portal vein; sinusoids empty only into the central veins. *Lower.* Rat. The area around large portal tracts receives its blood supply from remote small portal vein branches; free entrance of sinusoids into both central and sublobular veins. (Elias, H., and Popper, H.: *A.M.A.Arch.Path.* 59:332, Mar. 1955.)

may have undesirable effects. It may cause or aggravate centrilobular necrosis by contraction of the central veins. Portal vein and hepatic artery obstructions, especially if they occur simultaneously, may lead to similar effects, such as partial or complete ligation of these vessels, which in turn leads to hepatic coma [2710].

Comparison of the Vascular Distribution in Man and Rats. Differences in the distribution of vessels between man and animals are of importance in the explanation of the development of cirrhosis [1155]. In the rat, the portal vein branches larger than $200\ \mu$ in diameter in the middle-sized and larger portal tracts are conduct-

ing vessels which do not distribute blood into the surrounding hepatic territories [907] (Fig. 58, bottom). These territories around the middle-sized and larger portal veins receive their blood from small portal vein branches in other portal tracts and are, therefore, nonportal from a functional viewpoint [2710]. They are susceptible to the same changes, such as fatty metamorphosis or fibrosis, which occur in other nonportal areas, like the central zone of the lobule. Fibrosis in these nonportal zones produces fibrous connections between portal and central canals early in cirrhosis

formation. In man veins up to $280\ \mu$ in diameter discharge sinusoids into the surrounding parenchyma, and small portal vein branches accompany the larger conducting vessels into the portal tracts (Fig. 58, top). The parenchyma, therefore, even around the larger portal tracts, is portal in character.

In rats, sinusoids empty freely not only into central veins but also into all larger tributaries of the hepatic vein, whereas in man only the central vein, not the sublobular vein, receives sinusoidal blood.

LYMPHATIC VESSELS, TISSUE SPACES, INNERVATION, AND STROMA OF THE LIVER

LYMPHATIC VESSELS AND TISSUE SPACES

Structure

The communicating lacunar cavity system between the hepatic-cell plates is filled by sinusoids and by a system of intercommunicating tissue spaces which separate sinusoids from the hepatic-cell plates (Figs. 33, 35A). These spaces are drained by the lymphatic system, which starts with its smallest roots in the portal tracts and possibly to a small extent within the lobular parenchyma as well as in the hepatic capsule and empties into the large extrahepatic lymphatic vessels which drain the liver lymph into the cisterna chyli and the thoracic duct.

Tissue Spaces. Between the sinusoidal wall and the hepatic-cell plates, the space of Disse, traversed by argentaffin fibers, can be demonstrated by injection from the larger lymphatic vessels. It was originally considered a lymph space [340], but the results of injections are now considered to be artefacts, and the space which is not lined by endothelium is a tissue space. Its extracellular fluid is drained into the lymphatic vessels, which originate mainly in the portal tracts. Whether the space itself exists under normal circumstances or is an artefact resulting from shrinkage during fixation is unsettled. It has been found in animals by vital microscopy [1808] but not by electron microscopy [385]. In man, the space is invisible under normal circumstances in biopsy specimens, the sinusoidal wall being closely adherent to the hepatic-cell plate. In autopsy material, this space is not visible after instantaneous death. An agonal period of only a few minutes, as seen in death after hanging, drowning, or acute coronary occlusion, suffices to open it, and it appears filled with an

albuminoid material [2625] (Fig. 32). The extreme permeability of the sinusoidal wall during anoxia of only a few minutes' duration permits sufficient protein to pass through the sinusoids to exceed the absorptive capacity of the lymphatic vessels (see Structural Characteristics of the Hepatic Sinusoids, Chap. 14) and to produce edema, which opens the space. Unlike the tissue spaces in man, in most animals studied the tissue spaces do not open during the agonal period and are demonstrable only in frank edema [2625]. The escape of protein into the tissue spaces, a borderline pathologic condition, is discussed under edema of the liver (see Edema, Chap. 25).

The intralobular tissue spaces communicate with a larger tissue space, which extends between the limiting plate and the periphery of the portal tract. In dogs, rats, and pigs, this space of Mall is not lined by endothelial cells [2186] and is not injectable through the lymphatic vessels.

Intrahepatic Lymphatic Vessels. The smallest intrahepatic lymphatic vessels start in one of four places, with much species variation: (1) in the portal tracts; (2) in the central fields; (3) possibly in the parenchyma as small lymphatic vessels along arterioles and ductules; (4) in Glisson's capsule [907]. In man, the portal lymphatic vessels outnumber the central lymphatic vessels, whereas in the dog, many central lymphatic vessels are found [334] and contraction of the venous wall produces an opening of the surrounding central lymphatic vessels, not only by increasing the flow owing to the raised pressure, but also by mechanical suction by the wall of the vein [2625]. The central and peripheral distribution of lymphatic vessels does not differ much in rats and dogs [334]. Because of intercommunications the entire lymphatic system of the liver forms a unit.

PORTAL LYMPHATIC VESSELS. The endothelial lining of the lymphatic vessels transfers fluid as well as macromolecular substances probably from the space of Mall into the lymph vessels. The great permeability of the sinusoidal lining makes proteins and other large-sized particles, including bacteria, readily appear in the spaces of Disse and Mall. The number of lymphatic vessels in the portal field varies, several often being noted even in the smaller fields surrounding the other structures.

PERIDUCTAL LYMPHATIC VESSELS. The lymphatic vessels are closely associated with the bile ducts. In the small bile ducts, lymphatic vessels are found directly beneath the epithelium, suggesting a simple transfer of biliary material to the lymphatic vessels [334]. In the extrahepatic biliary ducts, the lymphatic vessels arise from a network in the muscular layer and from one in the subepithelial layer.

CAPSULAR LYMPHATIC VESSELS. In Glisson's capsule particularly, an extensive intercommunicating network exists [1932], which in turn communicates with the intrahepatic network and drains the subcapsular area. Extensive communications also occur with lymphatic vessels in the bed and wall of the gallbladder [2105].

GALLBLADDER LYMPHATIC VESSELS. The gallbladder has an extensive submucosal lymph plexus and a subserosal one. They communicate with each other and with the hepatic lymphatic vessels in the gallbladder bed. They are drained to the cystic or sentinel node at the neck of the gallbladder, which also receives drainage from the quadrate lobe of the liver.

Lymph Drainage. Individual as well as species variations exist in the drainage of lymph from the liver, and despite extensive studies, definite information is not available [183]. Three pathways of lymph drainage have been thoroughly studied in the dog [334, 462]. They probably also occur in man.

1. The bulk of the hepatic lymph coming from the interior of the liver collects into larger channels along the portal vein branches. It leaves the liver at its hilus via several valve-containing lymph vessels, grouped in two bundles at either end of the transverse fissure. These vessels extend to the hepatic lymph nodes in the lesser omentum along the course of the hepatic artery. The lymphatic vessels of the extrahepatic bile ducts, as well as of the gallbladder ducts, also enter the hepatic lymph nodes. From here the efferent lymph vessels

extend dorsally to a chain of celiac or preaortic nodes around the aorta and celiac axis, and thence to the cisterna chyli. Anastomoses exist with the duodenal, pancreatic, and gastric lymph nodes. In the presence of adhesions, direct anastomoses may exist between these lymphatic vessels. Some lymphatic vessels run directly to the thoracic duct from the hilus of the liver.

2. A few intrahepatic lymphatic vessels collecting around the central veins follow the tributaries of the hepatic veins. They collect around the proximal portion of the inferior vena cava and accompany the vena cava through its diaphragmatic hiatus. They enter the thoracic duct directly [334] or pass through nodes at the termination of the inferior vena cava. This pathway seems to be relatively unimportant in man.

3. Lymphatic vessels of the capsule and immediate subcapsular areas of the liver may collect either into channels which pass through the diaphragm anteriorly or posteriorly along the hepatic vein to enter the thoracic duct, or into channels which drain into the celiac nodes and the cisterna chyli.

Function

Amount of Hepatic Lymph. During starvation, the liver contributes from one-fifth to one-half of the thoracic duct lymph flow. The older data are based on investigation of thoracic duct lymph. Recently, methods have been described to cannulate the hepatic lymphatic vessels directly in the dog [462] as well as in the rat [336]. In the normal nonanesthetized dog, hepatic lymph amounts to nearly 0.2 ml per minute [462]. In the anesthetized dog the rate of flow is increased. In 200-gm rats on a mixed diet, approximately 5.0 ml lymph is obtained a day [336]. The rate of flow varies greatly, depending chiefly on hydration and state of digestion. In anesthetized dogs, the lymph produced by the liver equals about half the plasma volume [336].

In man, the thoracic duct lymph flow is about 1.0 ml per minute, or 1.4 ml per kg per hour [682]. The hepatic lymph flow is increased by intravenous administration of glucose and by ingestion of food, somewhat parallel to the increase of the thoracic duct lymph, which also drains the intestine [462].

Exercise does not increase hepatic flow as much as the flow from the intestine. Acute carbon tetrachloride intoxication in animals leads to blood lymph, with an increase in lymph flow [462]. In

experimental congestion or cirrhosis, the hepatic lymph flow may be increased to two to five times normal [2447]. After constriction of the thoracic portion of the inferior vena cava, the increase is even greater and the hepatic hilar lymphatic vessels become greatly dilated [3439].

The thoracic duct lymph is increased and sometimes contains erythrocytes in starving animals, in anaphylactic or peptone shock [2980], in portal obstruction, and in many intoxications [945]. This increase is greatest in the dog, in which contraction of the sphincters in the hepatic vein increases lymph formation by raising the venous pressure and possibly also by opening the lymphatic vessels in the central canals (Fig. 53, lower right). In general, the lymph flow is dependent upon the same factors in the liver as in other organs. It decreases with reduced arterial pressure and is apparently influenced by the same lymphagogues as are other lymphatic vessels.

Constitution of Hepatic Lymph. The protein concentration of the hepatic lymph is about 80 per cent of that of the plasma and is considerably higher than that of intestinal lymph [2447]. In 24 hours about 35 per cent of the circulating plasma protein passes through the hepatic lymph of normal dogs. The protein fractions are practically identical to those in serum except for a slight preponderance of albumin [591]. Hepatic lymph is also identical to the gallbladder lymph, another fact that emphasizes the close relationship between the two [2105]. In passive congestion produced by constriction of the inferior vena cava and in experimental cirrhosis, the protein concentration increases slightly but never reaches the plasma concentration. In absolute amounts, two to four times as much protein passes into hepatic lymph in congestion and almost six times as much in cirrhosis, in view of the increased total lymph flow [2447]. This explains the severe hypoproteinemia which rapidly develops in the presence of chronic lymph fistulas. In rats with cirrhosis, flow and protein concentration of the cisternal lymph are increased. The protein concentration of fasting thoracic duct lymph is increased by administration of intravenous fluid, by intoxications such as allyl formate intoxication [945], or by anaphylactic shock.

The concentration of total fatty acids in hepatic lymph is lower than that in intestinal lymph and is not influenced by feeding. The same is true for phospholipids and cholesterol, which are found in similar concentrations as in plasma [338]. Glu-

cose and chloride concentrations may exceed those in plasma, while inorganic phosphorus concentration is identical in both [462].

Ligation of the common bile duct almost immediately increases hepatic lymph [462]. In thoracic duct lymph, levels of bile acids and prompt-reacting bilirubin rise early, in contrast to the cholesterol level [1220]. Alkaline phosphatase activity increases to a lesser extent. Similarly, after carbon tetrachloride intoxication bilirubin and alkaline phosphatase activity increase parallel to the increase in plasma concentrations, whereas bile acid and cholesterol levels do not change [1220].

Production of Hepatic Lymph. Although the hepatic lymph drains the tissue fluid from the spaces of Mall and Disse, its constitution differs from that of the tissue fluid. In general, lymphatic vessels drain from the tissues particles or molecules, such as foreign material and insoluble proteins and fats, which can not be absorbed by blood capillaries. This drainage is initiated by phagocytosis by the lymphatic endothelial cells. These cells, which are mainly found in the portal tracts, localize inflammatory reactions caused by bacteria or other injurious substances that have passed through the tissue spaces into the lymphatic vessels.

PROTEIN IN HEPATIC LYMPH. The protein content of the tissue fluid in the liver is considerably higher than anywhere else in the body. This is in part the result of the increased permeability of the hepatic sinusoids and in part the result of transport of serum proteins formed by the liver.

Transport of proteins is one of the main functions of hepatic lymph [843]. Increased permeability of sinusoids or hepatic cells in passive congestion or hepatic damage may lead to accumulations of amounts of protein in the tissue spaces in excess of that which the lymphatic vessels can remove. The resulting edema has been considered an inflammatory process and termed "serous hepatitis" [945, 2797].

INNERVATION OF THE LIVER

Structure

INTRAHEPATIC NERVES. A fine network of intra-lobular nerve fibers connected with the hepatic cells as well as with the sinusoids has been demonstrated. This network is supposedly rather dense and follows the spaces of Disse. Extensive plate-like contacts with passage of the fibers through the

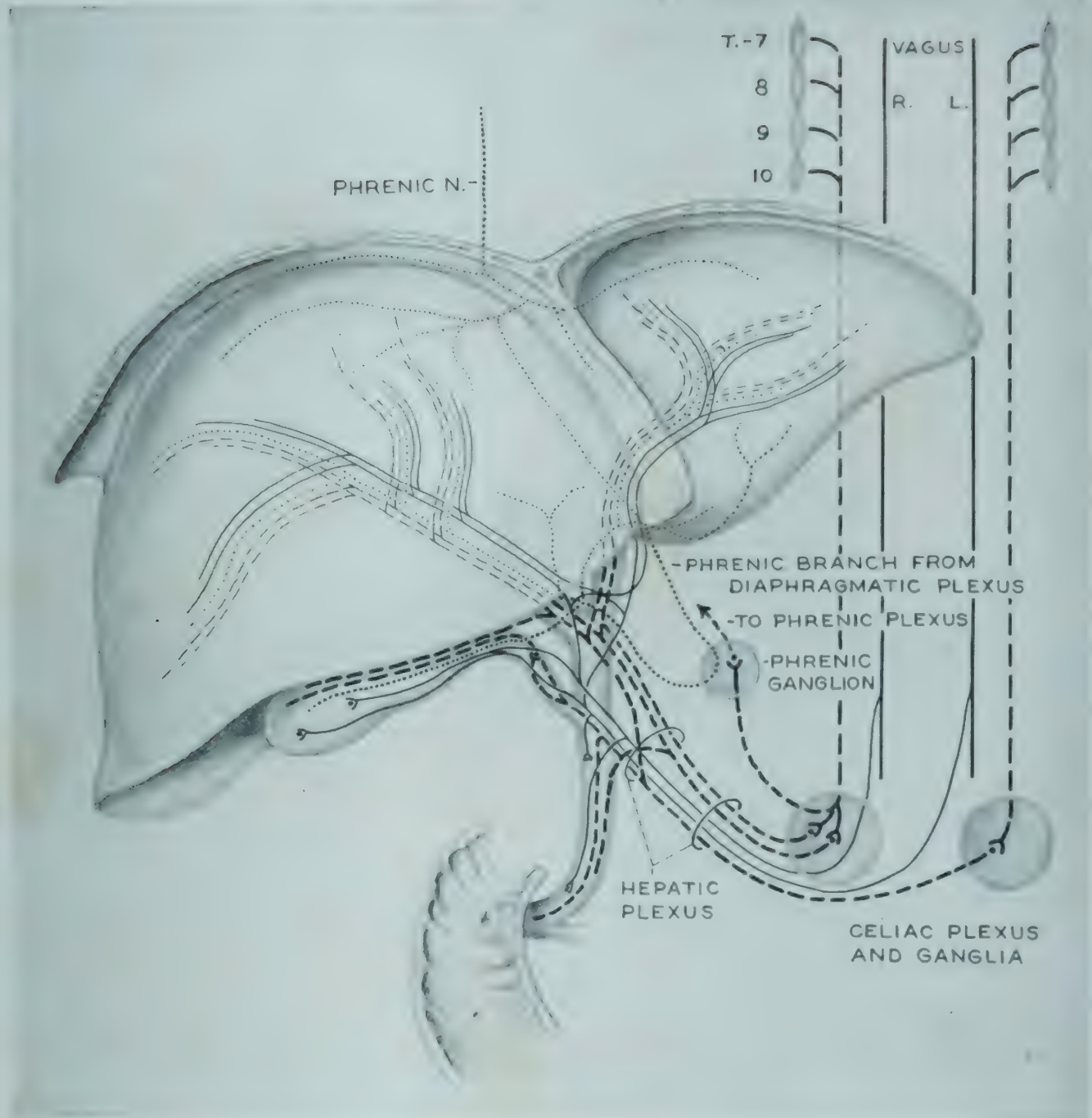


FIG. 59 Nerve supply of the liver. (Redrawn from Lewis, H. D.: *Ann.Int.Med.* 35:878, 1951.)

Kupffer cells have been described. The portal tracts contain small, nonmyelinated fibers occasionally intermixed with larger myelinated fibers [39, 1886]. The bundles generally follow the ramifications of the hepatic artery. In the wall of the bile ducts, a network of nerve fibers extends below the epithelium. This is conspicuous in the larger bile ducts, where groups of ganglion cells have also been described [2582]. In the central canals only a few fibers of sympathetic and parasympathetic origin have been demonstrated. The arterial innervation is exclusively sympathetic, whereas the bile ducts are innervated by both. Sympathetic

afferent fibers supposedly exist, but not parasympathetic ones [1981].

EXTRAHEPATIC NERVES. At the hilus of the liver, the nerves are arranged in two plexuses [1981] (Fig. 59). The anterior smaller one surrounds the hepatic artery. It consists of sympathetic postganglionic fibers coming from the left celiac ganglion. The preganglionic fibers originate in the seventh to tenth thoracic ganglia and travel with the left great splanchnic nerve. Parasympathetic preganglionic fibers from the right abdominal branch of the left vagus join this plexus. The anterior plexus provides the cystic duct and gall

bladder with part of its parasympathetic innervation and most of its sympathetic innervation. The parasympathetic synapses are within the walls of the bile ducts and gallbladder. The posterior plexus obtains its sympathetic fibers from the right celiac ganglion, which in turn receives its fibers from the seventh to tenth thoracic ganglions via the right great splanchnic nerve. Preganglionic parasympathetic fibers from the right vagus pass through the right celiac ganglion without synapsing and join the posterior plexus. The plexus lies behind the portal vein and supplies the lateral nerves of the gallbladder and the common duct with sympathetic and parasympathetic fibers. Its branches join with those of the anterior plexus to provide the intrahepatic innervation [1981]. Afferent fibers to the right phrenic nerve originate from the dome of the liver via the coronary and falciform ligaments and parts of the capsule. In addition, fibers from the right phrenic nerve enter both hepatic plexuses after passing through the phrenic ganglion to provide some afferent innervation for the gallbladder and liver. The sympathetic and parasympathetic nerves also carry afferent impulses. Fibers which carry pain stimuli follow the sympathetic nerves in addition to the phrenic nerve.

NERVES IN THE BILIARY TREE. The nerves to the gallbladder approach this organ with the vessels and extend into it in the subserous and subepithelial layers [573, 1401]. In the subepithelial layer, a fine nerve network sends terminal branches up to but not between the epithelial cells. Small fibers pass through the muscular layer. Outside this layer a dense plexus containing ganglion cells is seen, which is homologous to the Auerbach plexus of the intestine.

The innervation of the papilla of Vater originates from branches of the intestinal plexuses and the gastroduodenal nerve. The nerve supply of the sphincter of Oddi is both sympathetic from the splanchnic and parasympathetic from the vagus nerves. Numerous filaments come from the anterior and posterior hepatic plexuses and follow the common bile duct, ending in the papilla of Vater [91, 1077].

Function

The important role of autonomic nervous innervation in the biliary tract has been discussed as part of its function (see *Nervous Stimulation, under Interrelation between Gallbladder and Sphincter of Oddi*, Chap. 16).

The afferent innervation of the liver itself is complex. The presence of nerve fibers in the liver suggests an influence upon hepatic function and biliary secretion. The innervation of the intrahepatic blood vessels, important in the regulation of hepatic function by alteration of the blood supply, remains the most clearly understood, since it is similar to that of all other vessels.

HEPATIC PAIN. The route of the nervous impulses primarily transmitting pain sensations is not known. The main type of pain produced in the liver is dull pain, associated with diffuse tenderness on pressure over the liver and frequently with pain in the right shoulder. Occasionally, a beltlike skin segment limited by the ninth thoracic and first lumbar vertebrae on the right side of the body is hypersensitive. Pain often accompanies enlargement of the liver, especially if it is acute as in congestion, and has been associated with stretching of the capsule and traction on the hepatic ligaments. This and the associated shoulder pain suggest phrenic innervation. The peritoneal cover of the liver is not sensitive if touched during peritoneoscopy. During biopsy, sharp diffuse pain occurs if the surface is indented before perforation, while perforation itself may cause pain which radiates to the shoulder [1981]. This pain can be relieved by sympatholytic agents such as tetraethylammonium chloride [2892]. Moreover, lesions in the liver such as small abscesses, tumors, or areas of necrosis cause pain radiating even to the shoulder without stretching the capsule or ligaments and without inflammation of the capsule. This pain may be the result of inflammation or congestion in an otherwise insensitive organ [3644], the phrenic nerve probably being the pathway.

BILIARY TRACT PAIN. Another type of pain, sometimes associated with hepatic pain, is circumscribed tenderness in the gallbladder region, which results from edema of the gallbladder bed, usually associated with edema of the liver [945]. Colicky pain and sharp doubling-up pain result from contractions of the sphincter of Oddi. These pains radiate through to the back just below the tip of the right scapula, to the right shoulder, and to the substernal area and the anterior left chest. Another source of pain is increased biliary pressure leading to biliousness (see Chap. 16). Spasm of the gallbladder and biliary ducts results in colicky but not severe pain. Finally, peritoneal pain may result from involvement of the subserosa or serosa of the gallbladder and less commonly

of the liver. This pain is sharply defined, knifelike, and characteristically associated with hyperesthesia of the skin.

STROMA

The stroma of the liver is composed of four elements: (1) the hepatic capsule; (2) ramified trabeculae extending from the capsule into the parenchyma to serve as portal tracts, or canals, which contain the hepatic artery, the portal vein, and the bile ducts; (3) a narrow connective tissue ring in the central canal surrounding the tributaries of the hepatic veins; (4) the reticular framework of the liver, which extends between the portal and central canals and which is composed of fine fibers arranged around the hepatic cells.

Hepatic Capsule. In man, the hepatic capsule is much thicker than in many animals. In healthy adults, it is between 43 and 76 μ thick, whereas in animals, such as the pig, it is only about 10 μ thick [2582]. It consists of a dense network of interwoven, thick, collagenous fibers and membranes, between which elastic elements are irregularly distributed. On its outer surface, the capsule is covered by the serosal mesothelium. The deeper layers have a slightly looser texture and contain lymphatic vessels and a few blood vessels. The inner surface, which borders the lobular parenchyma, is sharply limited, except for radial fibers which firmly fix the capsule onto the parenchyma. Where the border between two lobules reaches the hepatic capsule, the parenchyma forms a groove in which the collagenous bundles of the interlobular zone merge with those of the capsule. In contrast to the arrangement found in animals, however, the capsule covers these grooves smoothly and without indentation. The capsular vessels communicate with branches of the portal vein but not with the hepatic vein. The capsule also contains aberrant bile ducts (see *Intrahepatic Bile Ducts*, Chap. 15). The capsule becomes thicker at the hilus, or porta hepatis, and around the inferior vena cava. From the hilus, the connective tissue of the portal tracts extends into the liver. The hepatic capsule, together with the portal tracts, is often designated as the capsule of Glisson. The thinner connective tissue of the central canals originates from the reinforcements of the hepatic capsule around the inferior vena cava. The capsule is also loosely thickened at the gallbladder bed, where it contains many vessels and nerves.

Portal Tracts. FIBERS. The portal tracts, originally called "interlobular spaces," consist of irregularly arranged collagenous connective tissue bundles, between which some fine, fibrillar reticulum fibers are suspended (Fig. 60, upper left and right). The dense connective tissue protects the vessels, which are firmly anchored within the portal tracts. Elastic fibers or membranes originating from the elastic lamellae of the portal vessels are interwoven. The structures of the portal tracts show species differences. In man, the adventitial layers of the arteries and veins seem to unite. The interlobular bile ducts are close to the vein or separated from it by lymphatic vessels. In the larger portal tracts many lymphatic vessels are found. The tissue of the portal tracts forms 4.6 per cent of the total volume of the liver in the adult, and 6.0 per cent in children [2582]. In man, the portal tracts form trabeculae with broad meshes of parenchyma between them. In the pig, they form complete capsules around each lobule. This, however, is not necessarily associated with an increase of the total portal tract connective tissue, since the connective tissue in the pig is much thinner around the vessels [2582]. In man, narrow secondary trabeculae extend into the parenchyma from the portal tracts. They contain the intralobular branches of the hepatic artery or precapillary arterioles, the intralobular ductules, and sometimes small intralobular lymphatic vessels.

CELLULAR ELEMENTS. Between the connective tissue bundles, cells with large, vesicular nuclei are found. Their cytoplasm may contain engulfed material, often pigment. Some of these cells are fixed histiocytes, others are wandering monocytes. Their origin from Kupffer cells has been repeatedly claimed [2582]. The pigment-carrying cells in the livers of cold-blooded animals are in the same category [1659]. Some lymphocytes and plasma cells may also be present. These cellular elements are frequently arranged around the lymphatic vessels (Fig. 60, lower left). The incidence of the accumulation of histiocytic and lymphocytic cells varies in apparently normal persons in the same liver and in different livers. They become larger and more common with increasing age. All transitions, from only a few cells to large aggregations which form lymph follicles with germinative centers [1735] or to irregular accumulations without any pattern, are encountered, without evidence of a significant disorder of the liver. For instance a slight increase in portal cellularity was seen

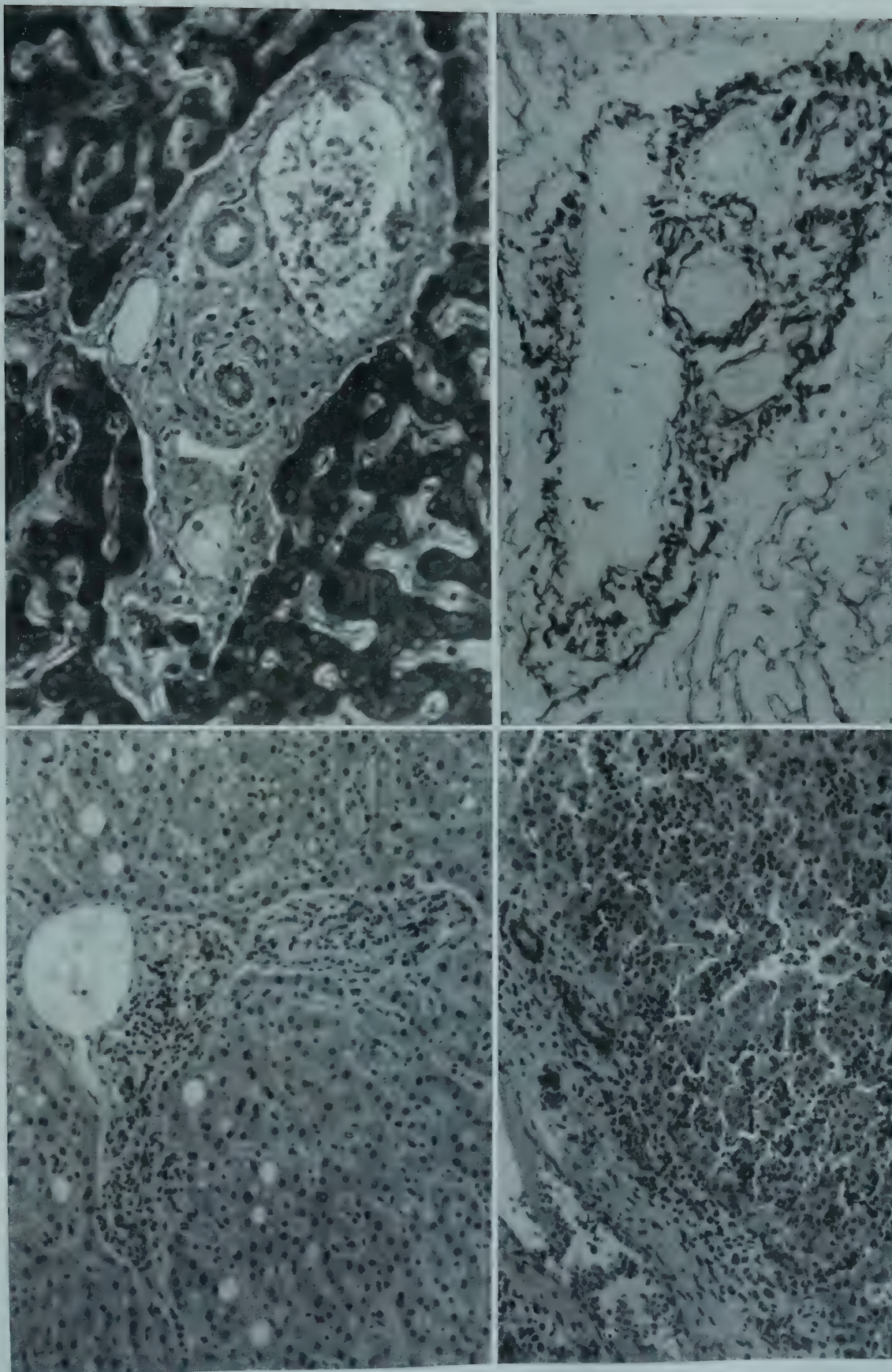


FIG. 60 *Upper left.* Normal portal tract with bile duct. Trichrome stain ($\times 220$). *Upper right.* Normal portal tract. Thick collagenous fibers, appearing comma-shaped in the section, surround vessels in the portal tract. Silver impregnation ($\times 140$). *Lower left.* Lymphocytes and histiocytes in a portal tract of a normal liver. H&E ($\times 120$). *Lower right.* Liver of premature infant of 7 months' gestation. The portal tract contains a large number of hematopoietic cells. Small groups of these cells are also found in the parenchyma. H&E ($\times 130$).

in approximately 25 per cent of healthy young soldiers dying instantaneously, and considerable infiltration, often with segmented leukocytes in one or another portal space, was noted in 40 per cent of this group [2626]. Differentiation from pathologic processes is difficult, since the possibility of some injury to the liver, such as from toxic material absorbed from the intestine, can not be excluded. In general, the presence of infiltration in some portal tracts probably has little significance. Its occurrence in almost every portal tract seems to be abnormal [1735]. In liver biopsy specimens, if such infiltrations are conspicuous, the term "nonspecific reactive hepatitis" is applied (see section of that title in Chap. 41). In embryonal life and in premature infants, hematopoietic foci are found in the portal tracts, and they may persist for several months in various pathologic conditions.

In mice and guinea pigs, infiltrations appear after splenectomy [2582]. They have been also produced by x-ray irradiation, intoxications, and injection of trypan blue.

Central Canals. The connective tissue around the central veins and other tributaries of the hepatic vein is dense, although sparse in quantity. A loose adventitia is not found in man or most mammals. The wall of the vessel is directly adjacent to the lobular parenchyma and is anchored there by radial fibers which extend through the parenchyma to the portal tracts. This close attachment prevents collapse of the veins from the negative pressure in the pleural cavity that is directly transmitted into these vessels. The radial fibers originate from the longitudinal fibers of the venous wall.

Intralobular Framework. This consists of a few collagenous fibers, radially arranged, connecting the central fields with the portal tracts, in addition to a supporting framework of fine reticular fibers, which are poorly and irregularly stained by Van Gieson's stain and by Mallory's aniline blue stains. The reticulum framework is demonstrable by silver impregnation, in which reduced metallic silver is precipitated on the fine argentaffin fibers, rendering them visible under the microscope. The network forms the supporting structure of the sinusoids (Fig. 32*B, D*). It consists of longitudinal and cross fibers. The longitudinal arrangement is the main component of the intralobular framework. These longitudinal fibers remain in contact with the sinusoidal wall. Transitions between them and the collagenous radial fibers exist. The cross fibers originate from the longitudinal fibers, and run in an arcuate course to the hepatic plates, being attached to them. They are well seen only with expanded tissue spaces, and they seem to merge with the longitudinal fibers in most biopsy specimens or in animals [2625]. Near the portal and central fields the arcuate fibers merge with the reticulum fibers of these structures. The fibers are probably the product of the sinusoidal endothelial cells, similar to the reticulum fibers of lymph nodes or spleen, which are formed by mesenchymal elements of the reticuloendothelial system [2582]. Recent electron microscopic studies on reticulum fibers indicate that they have a characteristic segmentation and differ from the elastic fibers and collagenous fibers. They may be precursors of collagenous tissue. The argentaffin fibers of the liver do not give so distinct a PAS reaction as in other organs [2157].

Certain structural and functional alterations concern the liver as a unit and not the individual structural entities thus far discussed. The question of the liver lobule requires consideration before the gross anatomy is discussed. Embryology, malformations, and postmortal changes are also discussed in this chapter.

THE LIVER LOBULE

Grossly, on the cut surface of the normal liver, a pattern of red dots representing central areas is seen against a brown background, which is interrupted by gray areas representing portal tracts. The distance between red dots varies from 0.5 to

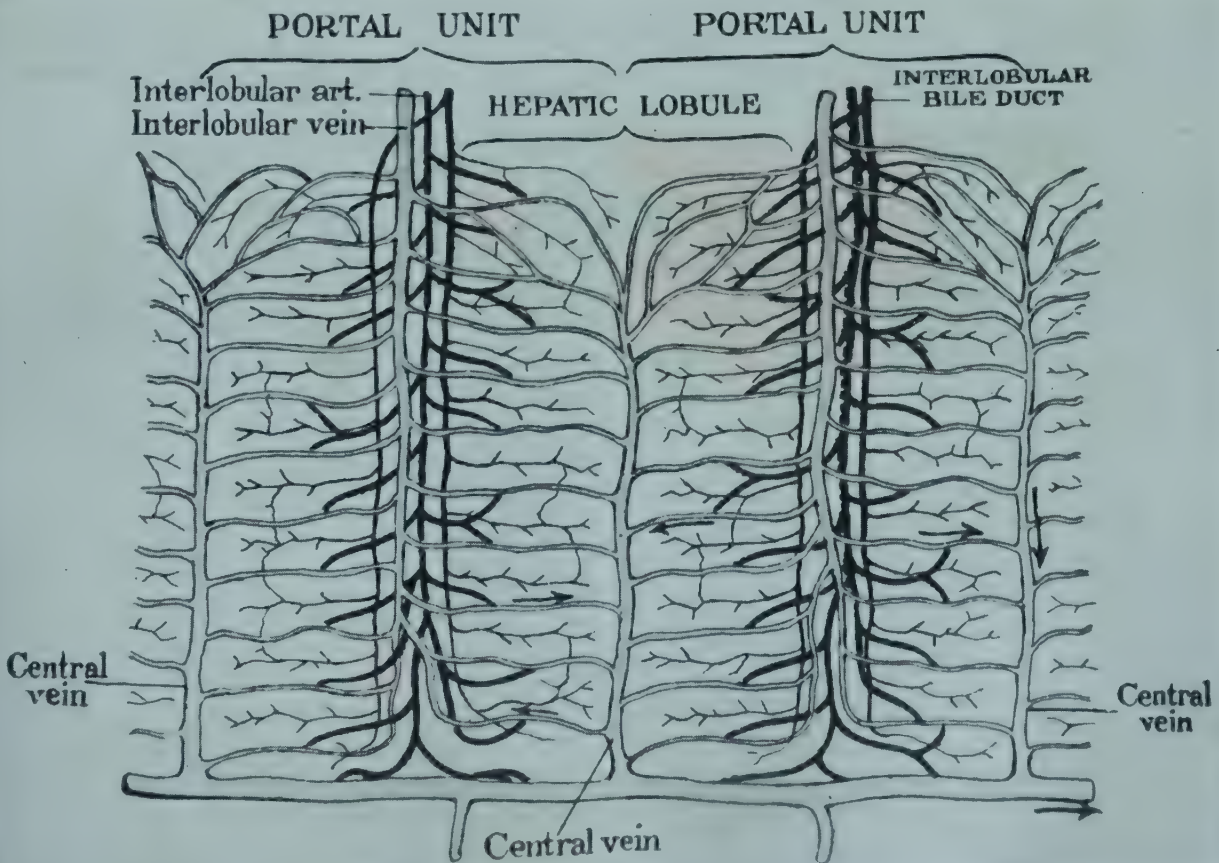


FIG. 61 Diagram of the portal unit and vascular relations of the hepatic lobule (after Szymonowicz). (From Schaeffer, J. Parsons, ed.: *Morris' Human Anatomy*, 10th ed., New York, Blakiston, 1942.)

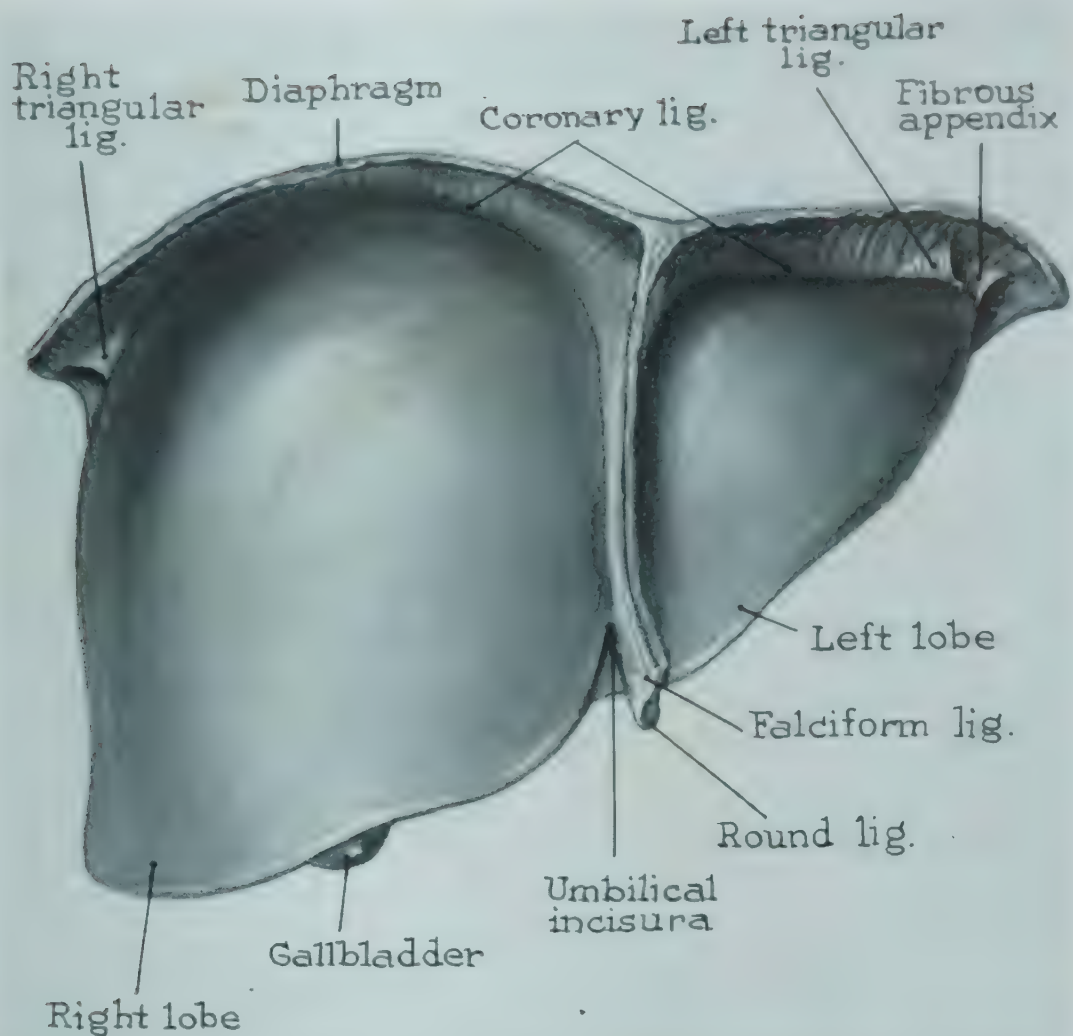


FIG. 62A Ligaments and anterior surface of the liver.

2.0 mm. This is the diameter of the hepatic lobule. Its form, shape, and even existence have been the subject of a long-standing controversy.

PRINCIPLE OF THE LOBULE. Parenchyma is everywhere, interposed between the ramifications of the portal vein, i.e., in the portal tracts, and the hepatic vein, i.e., in the central fields. The portal tracts and central fields cross each other in space (Fig. 54). The division of the intervening continuous parenchymal mass depends upon interpretation rather than observation. The hepatic-cell plates normally converge toward the central vein. The generally accepted concept arranges the lobule around the central vein, the structures of the portal tract being boundary markers of the periphery. Another concept places the periportal field with the draining bile duct in the center of the lobule, i.e., the portal unit [2186], or portobiliary lobe (Fig. 61). The choice of units depends primarily upon whether the influence of the liver on the blood stream or the formation of bile is

accorded predominance among the hepatic functions.

PORTAL UNIT. Many earlier investigators, including Sabourin, Mall [2186], and Loeffler, have favored the recognition of a portal unit. Recently, spaces in the hepatic parenchyma, which become conspicuous in edema, were thought to demarcate portal units [2489].

CLASSIC UNIT. The classic liver lobule was defined by Kiernan as early as 1833. Much of the original support for the classic unit comes from studies on the adult pig liver, which has an almost complete connective tissue capsule around each lobule [884]. In all other animals, with the possible exception of the seal, the appearance of a lobule is produced by the convergence of the hepatic-cell plates toward the center. This convergence is mechanically produced by the blood flow in the sinusoids. In postnatal life, blood is sucked from the sinusoids into the central vein by the right heart. In the fetus, in which no nega-

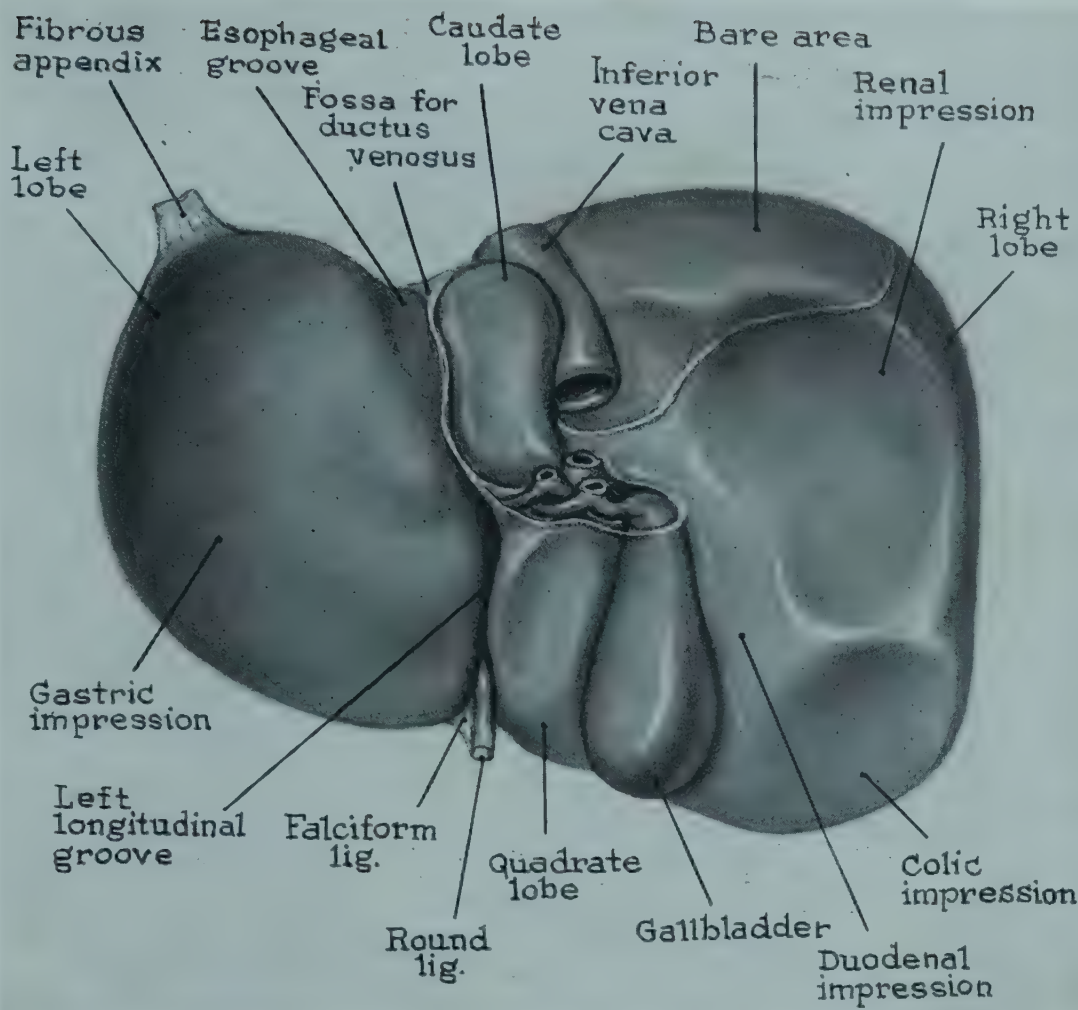


FIG. 62B Ligaments and inferior surface of the liver.

tive pressure exists in the thoracic cage, lobule formation is absent [2582]. The convergent arrangement of the hepatic-cell plates is therefore not fixed but results from the blood pressure gradient between the portal and central veins. Alteration of this gradient, as in congestion, reverses the lobular pattern. The classic liver lobule is thus a clearly apparent but poorly demarcated hepatic territory [907].

SHAPE OF LOBULE. The liver lobule is reported to be about twice as long as it is wide and to vary considerably in shape [2582]. Tangential branches of the terminal ramifications of the portal vein extend from the portal tracts along the periphery of the lobule and give rise to radial capillaries. These venous ramifications represent a functional vascular septum between lobules not apparent in routine histologic sections. These vascular septums correspond to the connective tissue septums in the adult pig. Since they are not uniformly arranged, the lobules have irregular shapes. In adults, the

distance between terminal portal vein branches and initial hepatic vein branches is about 0.3 mm; it is somewhat less in children. This distance is the radius of the lobule. Local changes in blood pressure and angulation of the septums cause variations in its width and shape. The vascular septum is absent in some parts of the circumference of the lobule, especially near the twigs of the branching central vein. This results in odd-shaped multiple lobules, which are common in the human liver. Moreover, each portal canal is surrounded by a limiting plate, which is itself surrounded by a peripheral sinusoid. From it radial sinusoids extend to the central veins of several lobules, thus obscuring the unity of the lobule [907]. Cytologically, at the periphery of each lobule, the mitochondria are larger and shorter, the Golgi apparatus is heavier, more glycogen is deposited, and less fat is stored [752]. Whatever the nature of the lobule, no part of the normal hepatic parenchyma is further than 1.0 mm from a portal tract, guaran-

teeing adequate blood supply and bile drainage to all areas.

GROSS ANATOMY

Lobes and Surfaces. The liver is a pyramidal-shaped organ with its apex to the left and its base to the right (Fig. 62); the sides forming the anterior, posterior, superior, and inferior surfaces. It consists of a much larger right lobe, which occupies the entire right hypochondrium, and a smaller left lobe in the left hypochondrium, which is tongue-shaped, its sharp apex reaching the dome of the left diaphragm. It continues into the fibrous appendix of the liver. Some variation in shape may result because of skeletal deformities, such as kyphoscoliosis, in which the liver may be either a short wide organ stretching across the entire upper abdomen or a tall narrow one occupying the right half of the abdomen. Both lead to unusual findings on palpation or percussion.

The division into two lobes is not necessarily a functional one, since the left part of the right lobe is served by tributaries of the left branches of the hepatic duct and the portal vein. On the inferior surface, two small lobes are separated from the left portion of the right lobe by the grooves produced by the inferior vena cava and the gallbladder. The left half of the liver is interposed between the diaphragm and the antral and fundic portions of the stomach. The anterior and superior surface of the liver is convex, to fit in the concavity of the diaphragm; the upper pole reaches to the fourth or fifth intercostal space anteriorly. A small part of the anterior surface is in contact with the anterior abdominal wall. The posterior surface is almost vertically directed and is in contact with the lumbar portion of the diaphragm, the esophagus, and inferior vena cava. The concave inferior surface is in contact with the right kidney and adrenal gland, the transverse colon, duodenum, and stomach. Anteriorly, the inferior and superior surfaces meet in a sharp edge, which becomes slightly blunted in its right and also in its lateral aspects.

Projections of the Liver. The right lobe of the liver is covered by the rib cage in its lateral aspect (Fig. 63). The liver reaches to the tenth or eleventh rib in the midaxillary line in the upper right position. Usually the anterior edge crosses the costal arch in the midclavicular, or lateral body, line. In the epigastrium the liver is not covered and extends about three finger breadths

below the base of the xiphoid process in the midline. Since pleura and lung are interposed between the diaphragm and the right chest wall, a dull percussion tone is obtained over the upper third of the liver. The projection of its middle third is flat on percussion, since only pleura is interposed, not lung. Over the lowest third the percussion is also usually flat, except when intestinal loops between liver and anterior abdominal wall cause tympany. The border between dullness and flatness normally moves with respiration. The zone of flat percussion permits an estimation of the size of the liver, which is best appreciated when the subject is in the horizontal position.

Ligaments. The liver is covered by peritoneum except at the gallbladder bed, at the hilus, and at the adjacent portion of the posterior and inferior surfaces surrounding the groove of the inferior vena cava and continuing into a triangular area to the right of the inferior vena cava. The triangular bare area is in direct contact with the inferior vena cava, the right adrenal gland, and the diaphragm, to which it is connected by connective tissue strands containing the veins of Retzius, which are portosystemic anastomoses. This bare area is delineated by the upper and lower layer of a transverse peritoneal duplication, the coronary ligament. It extends to the right and left edges of the liver. Its free lateral margins are called the "triangular ligaments," the left one surrounding the fibrous appendix. Another serosal duplication, sagittal in direction, arises from the bare area to the left from the inferior vena cava. This falciform ligament extends to the diaphragm and reaches forward to an incisura at the anterior margin of the liver. The anterior free edge of the falciform ligament is reinforced, to form the ligamentum teres, which runs from the umbilicus to the incisura of the liver and which carries the fetal umbilical vein or its postnatal remnants.

From the porta hepatis and the venous ligament, the lesser omentum originates. This extends to the lesser curvature of the stomach and its continuation in the first portion of the duodenum. In the reinforced free edge of this omentum, the hepatoduodenal ligament, the large vessels run to the hepatic hilus.

Surface Contour of the Liver. The anterior and superior surfaces of the liver are usually smooth. The inferior surface is traversed by two longitudinal grooves, linked in the middle by a transverse groove. The transverse groove is the hilus of the liver, where the portal vein and hepatic

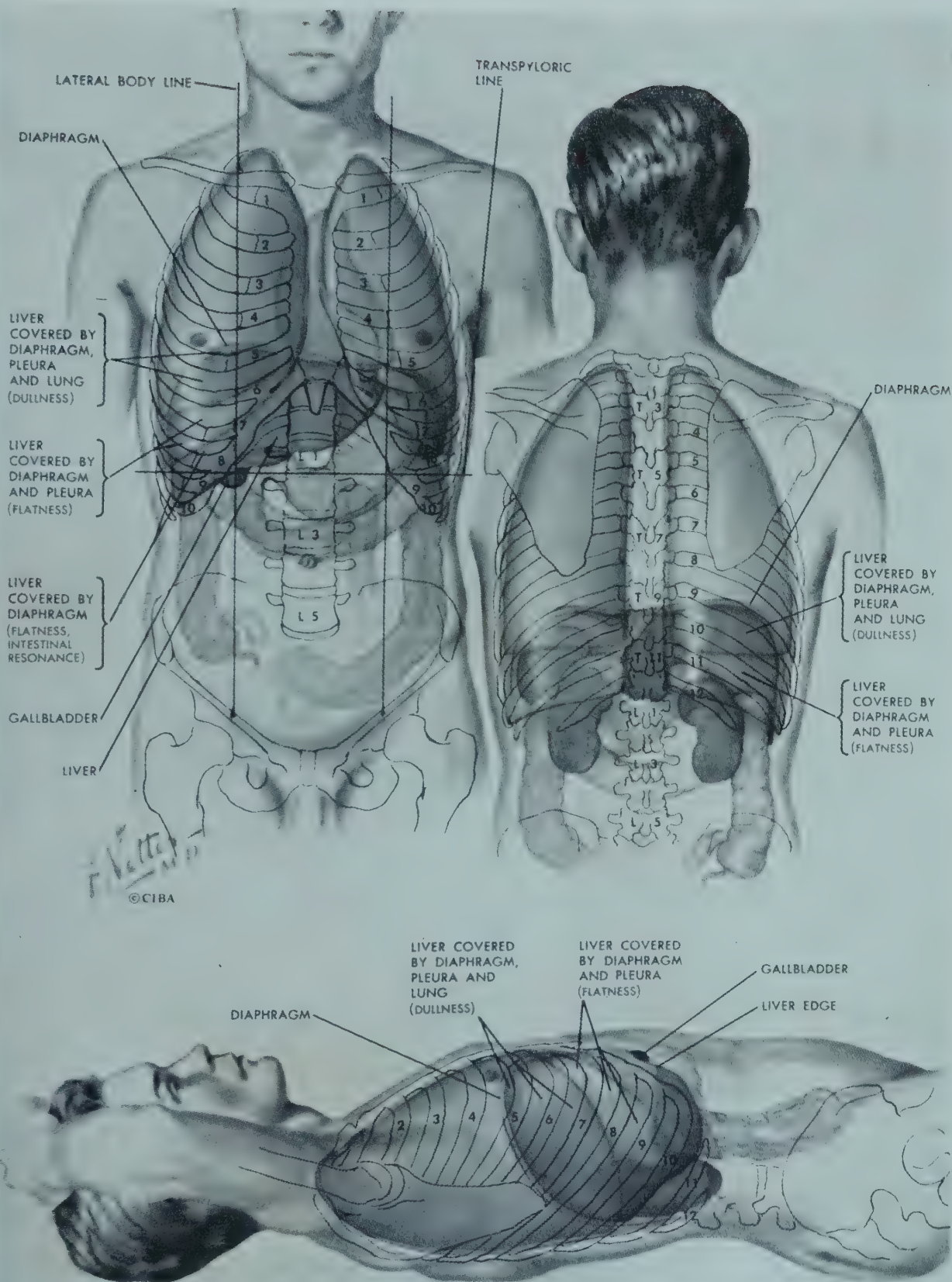


FIG. 63 Topographic relations of the liver. (Copyright © The Ciba Collection of Medical Illustrations, Frank H. Netter, M.D.)

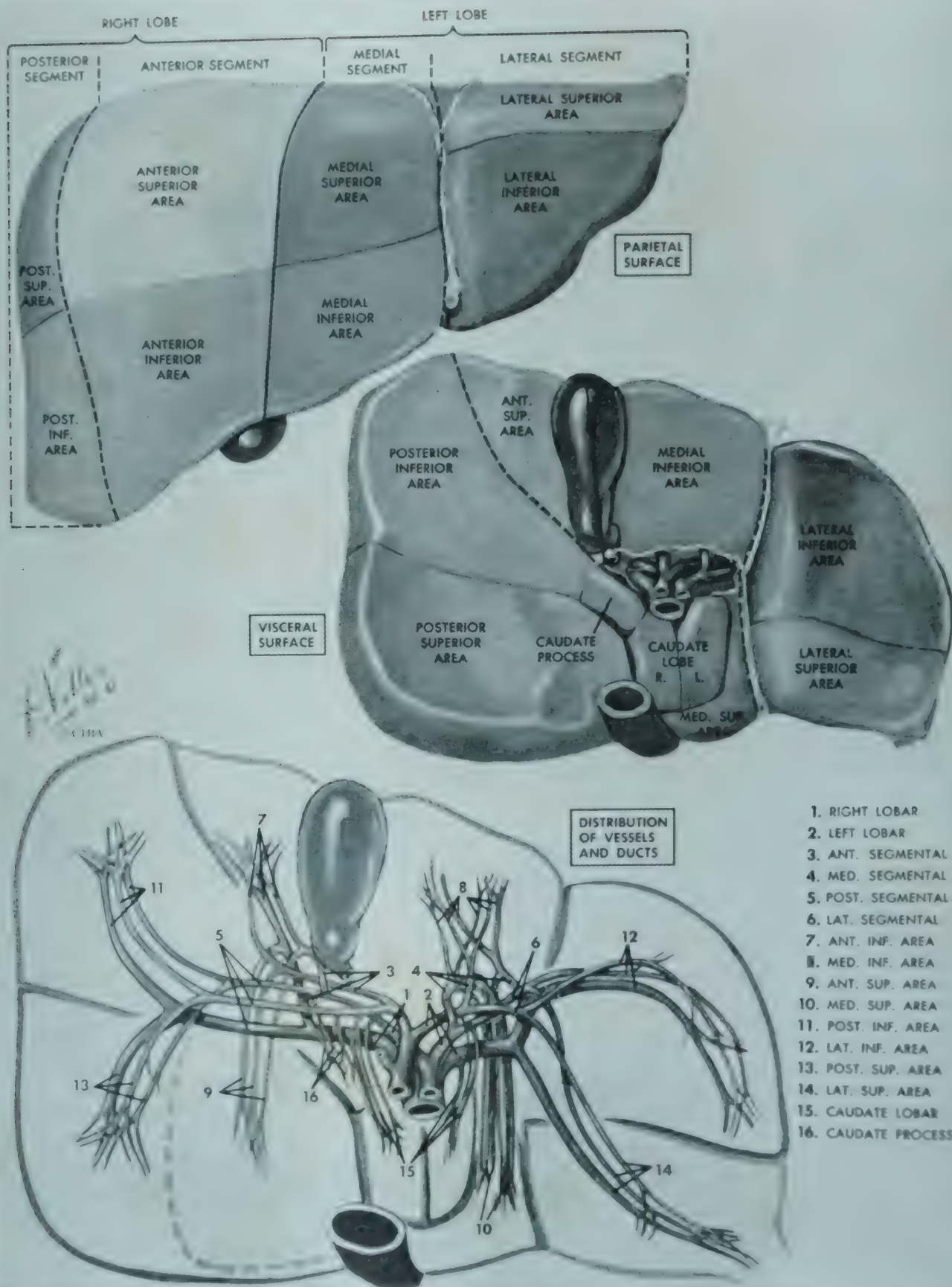


FIG. 64. Segments of the liver. Copyright © The Ciba Collection of Medical Illustrations, Frank H. Netter, M.D.

artery enter and the hepatic duct leaves the liver. The left longitudinal groove, which separates the right and left lobes, contains the continuation of the ligamentum teres of the liver, extending to the porta hepatis in the form of a strand. The posterior part of this groove harbors the remnants of the fetal ductus venosus Arantii, a fibrous strand in the adult called the "ligamentum venosum." The right longitudinal groove is interrupted in its middle portion by the protuberance of the caudate lobe. Its anterior part serves as the gallbladder bed. The posterior part surrounds the proximal part of the inferior vena cava, which is held in the groove by a fibrous strand, the ligamentum vena cava.

The plastic character of the hepatic tissue explains impressions of the neighboring organs on the surface of the liver. The convex posterior surface has only occasionally impressions produced by ribs. The inferior surface of the right lobe reveals a molded bas-relief (Fig. 62A, B). In addition to the gallbladder groove in the anterior portion and a very deep groove around the vena cava inferior in the posterior portion, flatter impressions are produced by the kidney, colon, duodenum, and right adrenal gland. In the posterior aspect of the left inferior surface, the esophagus produces a groove and the stomach frequently makes an impression. The development of the bas-relief shows individual variations, and some indentations border on malformations (see Indentations, under Malformations, later in this chapter).

Segments. Division of the liver into a right and left lobe usually follows the gallbladder-vena caval line, the right longitudinal groove. This gross anatomic division is justified in principle by the streamlines of flow in the portal vein (see The Portal Vein, Chap. 17).

The recently expanded interest in surgical resection of portions of the liver has stimulated gross anatomic studies about the distribution of the larger branches of the vessels and bile ducts [672, 909, 1437, 1438]. From these studies, segmental territories of the liver have been constructed, similar to the segments of the lung. From the point of view of the hepatic vein, the right lobe can be divided into a right and a dorsal part [1810]. Segmentation has also been based upon the distribution of the branches of the portal vein, hepatic artery, and bile ducts, which run parallel. In injection preparations a lobar fissure is apparent but is not seen on the surface. It extends from the gall-

bladder fossa inferiorly to the inferior vena cava fossa. This fissure is traversed by a hepatic vein trunk, and it divides the liver into a right and a left lobe, not identical with the usually accepted surface divisions. The vascular distribution subdivides the right lobe into anterior and posterior segments and the left lobe into medial and lateral segments. The lateral segment is usually recognized as the left lobe.

Each segment is further divided into a superior and an inferior area [1437] (Fig. 64). The hepatic vein ramifications often do not parallel those of the other vessels, and therefore excisable segments may be much larger; for instance, the entire right lobe has been considered one segment [907]. The concept of hepatic segments is still to be tested by surgical experiences.

Weight of the Liver. The weight of the liver represents one-fiftieth of the total body weight at almost all ages. It varies in male adults between 1,400 and 1,600 gm, and in female adults between 1,200 and 1,400 gm. In adults it measures on the average 25 to 30 cm in width, 12 to 21 cm in length, and 6 to 9 cm in thickness. In newborn children its weight averages 78 gm; at three months, 140 gm; at one year, 288 gm; at three years, 418 gm, and at twelve years, 936 gm [658].

EMBRYOLOGY

The development of the liver, which was apparently well established years ago on the basis of a series of classic studies [317, 2538], became problematic in view of recent embryological reinvestigations [907, 1545, 2021, 3247]. Originally the epithelial elements of the liver were considered entodermal in origin, derived from the foregut. Now, not only the mesenchymal elements but even part of the human epithelial liver plates are thought to originate from the mesothelial lining of the coelomic cavity [907]. Such a possibility is supported by the observation that hepatic cells are transformed into histiocytes in tissue cultures [1083]. The role of mesenchymal elements in the formation of epithelial hepatic-cell plates varies in different species. In some animals, like the guinea pig or chicken, they are derived only from entodermal elements; in some amphibians they are formed only from mesodermal elements, even the bile ducts arising from these mesodermal masses, which assume an epithelial appearance and connect only secondarily with the gut [907]. Future investigations will have to confirm the dual

—entodermal and mesodermal—origin of the hepatic parenchyma. The liver in amphibians develops by transformation of the yolk sac; the mammalian liver also develops in close relation to the yolk sac or vitelline vessels. It appears to serve as a substitute yolk sac, into which the mother deposits nutrients through the umbilical vessels during intrauterine life, while in postnatal life the organism does the same through the portal vein [907]. The concepts of hepatic embryology assist in the understanding of various pathologic processes such as bile duct proliferation, hepatitis, intrahepatic cholestasis, cholangiolitis and hepatic hamartomas, and cancers of the liver.

Hepatic Anlagen. The human liver develops from two or three anlagen [907] (Fig. 65). The first is the so-called "hepatic diverticulum," which is derived from the entoderm. It develops from a glandular layer in the ventral floor of the foregut, which develops in embryos 2.5 mm in length, near the origin of the yolk sac corresponding to the future duodenum [907]. From its caudad portion the extrahepatic bile ducts develop, with the gallbladder and cystic duct as a side arm. The ventral pancreatic bud may arise from the duodenum below the hepatic diverticulum; subsequently the pancreatic and common ducts have independent openings. It may originate from the gallbladder part of the diverticulum; subsequently a common ampulla develops for both ducts [2769]. The cephalad portion is composed of entodermal cell masses, which develop into several-cell-thick hepatic-cell plates and discontinuous cell masses with indistinct borders between epithelial cells and surrounding parenchyma [907, 2021]. The second anlage is a mass of splanchnic mesoderm in the primitive diaphragm, the transverse septum. Its upper part eventually develops into the true diaphragm, while the lower part is the ventral mesentery of the intestine. It serves as the site of formation of the serosal capsule and the stroma of the liver. In the septum transversum, a capillary plexus of isolated endothelial vesicles subsequently connects with the vitelline or omphalomesenteric veins, which also divide into small branches [2021]. The entodermal hepatic-cell plates and masses invest the vascular plexus of the vitelline vessels. Invasion of the plexus, as originally claimed, does not occur [907, 2021]. Apparently contact between the entodermal elements and the cells of the capillary plexus is the mutual stimulus for the specific differentiation into the hepatic-cell plates and the sinusoidal labyrinth,

with its Kupffer cells. This differentiation is presumably the mutual effect of organizers produced by the epithelial and mesenchymal tissue [3151]. The plates quickly become reduced to one cell in width. Hepatic tubules or cords, previously described [317], do not develop. Embryonal liver grows by simultaneous formation of new sinusoids and plates.

The supposed third anlage, developing posterior to the first entodermal anlage, is a cell mass derived from the coelomic mesoderm [907]. It enters the capillary meshes and is transformed into epithelial cells, which intermingle with epithelial cells of entodermal origin to develop into hepatic-cell plates. According to this concept, the epithelial portion of the anterior part of the liver is entodermal, the median portion entodermal and mesodermal, and the posterior portion only mesodermal [907]. This supposed third anlage would indicate the great potentiality of organizers in stimulating the transformation of mesodermal and entodermal cells to form the same end product, the epithelial hepatic cell.

Bile canaliculi differentiate in embryos 10 mm in length in the thick, almost cordlike hepatic-cell plates suspended in the meshes of the sinusoidal plexus [317] (Fig. 66). Bile secretion [3247] and accumulation of glycogen and fat [3224] start only after the third month of gestation. From the second to the seventh month of fetal life, islands of hematopoietic tissue abound between the endothelial lining and the hepatic parenchymal cells and within the sinusoids.

Bile Duct Development. The intrahepatic bile ducts are noticeable in 10-mm embryos, but active proliferation starts only in embryos 23 mm in length [1545]. In the intervening time, blindly ending segments are noted [2456], with an excess of intrahepatic ducts, which later seem to correspond to the ductules. Their epithelium may be ciliated. Some assume that the epithelium of the intrahepatic bile ducts develops into the hepatic cells, but most investigators feel that the hepatic cells and ductules are derived independently from the larger bile ducts and unite with them only secondarily [317]. For instance, some congenital atresias of the intrahepatic, or interlobular, bile ducts are associated with atresias of the extrahepatic ducts, while the ductules, or perilobular ducts, are intact [2156].

Even the origin of the intrahepatic bile ducts from the extrahepatic bile ducts [317] has been challenged on the basis of reconstruction studies.

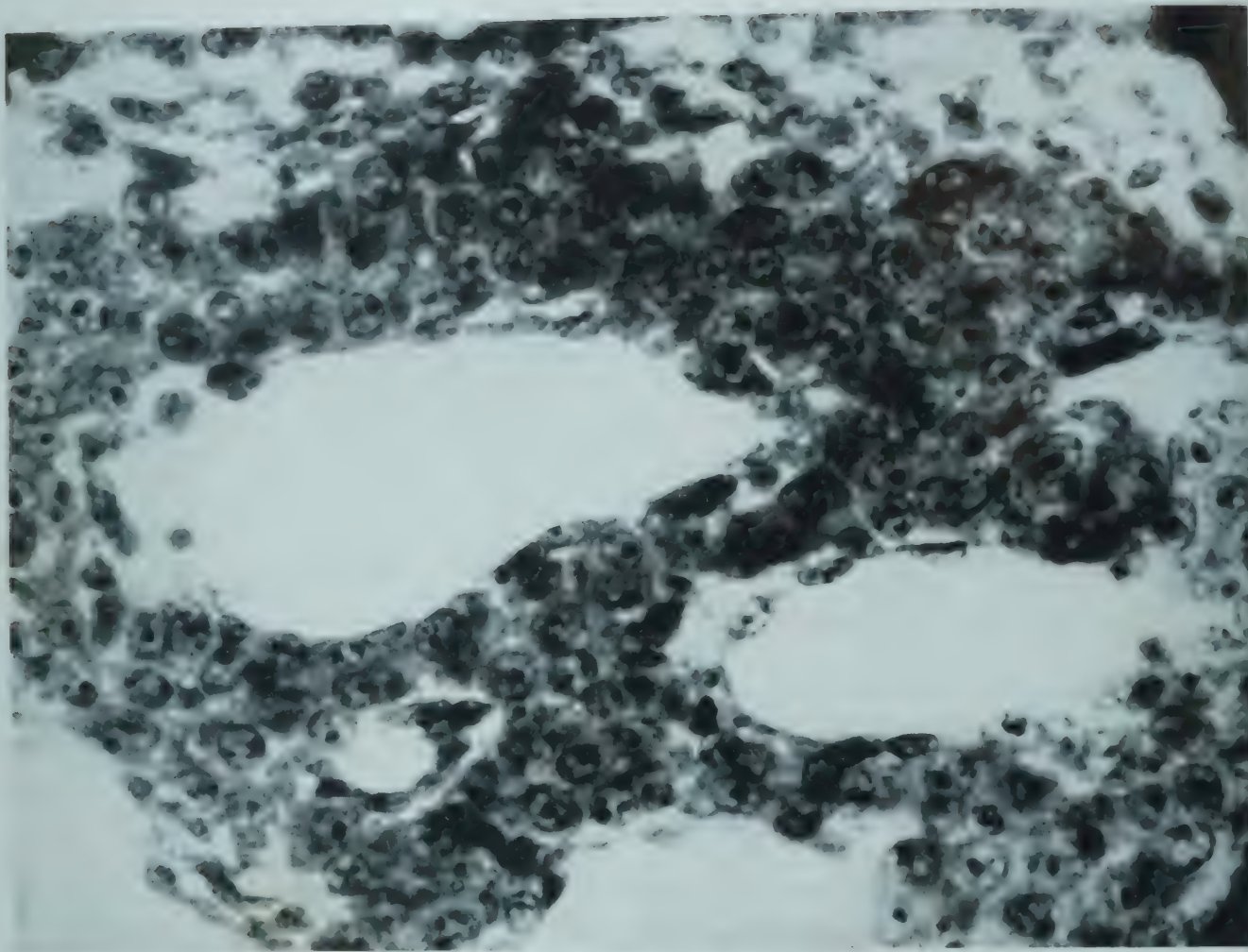


FIG. 66 Invasion of septum transversum by epithelial cells arranged in several-cell-thick plates around vitelline capillaries in human embryo with 27 pairs of somites. (*Courtesy of Dr. H. Elias.*)

These indicate that the entire intrahepatic bile duct system is derived from hepatic cells. In this development the effect of organizers again seems to be of major importance [2335].

STRUCTURAL ORGANIZERS. The stimulus for the formation of ductules from the hepatic cells and their further differentiation to bile ducts depends upon the contact of the hepatic cells with portal connective tissue and upon the circulation [317, 1545]. In tissue cultures hepatic epithelial elements adjacent to mesenchyme have been observed to assume bile duct characteristics [811]. Hepatic cells are transformed into bile duct epithelium in different parts of the liver up to the time of birth. This takes place near afferent veins (near smaller ones in older embryos) [1545]. Contact of entodermal and mesodermal cell masses with the capillary plexus stimulates the development of the hepatic cells. Similarly, contact of hepatic cells with collagenous connective tissue stimulates their transformation into ductules and ducts [586, 811, 1545]. The more extensive this contact, the more advanced the development;

complete development of the epithelial cells stimulates bile duct formation. The apparent dependence of the development of the bile ducts upon the diameter of the afferent, mainly portal, vessels has been explained by the influence of the pressure in the veins above the systemic venous pressure, owing to narrowing of the ductus venosus Arantii [1545].

SIMILARITIES BETWEEN EMBRYONAL DEVELOPMENT AND REGENERATION. The concept of the development of ductules and ducts from hepatic cells under the stimulus of contact with connective tissue and increased vascular pressure is important for the understanding of the so-called "ductular proliferation" (see Ductular Proliferations, Chap. 16).

Development of Hepatic Circulation. In embryos 4.5 mm in length, the paired vitelline, or omphalomesenteric, veins from the yolk sac are interrupted by a capillary plexus in the hepatic anlage in the septum transversum (Fig. 65). The cephalad part of the pair enters the sinus venosus of the heart together with the paired un-

bilical veins from the placenta and the common cardinal veins from the body of the embryo. The close relation of the umbilical and cardinal veins at this early period explains the portosystemic anastomoses that abnormally persist into adult life [565]. As the embryo grows, three wide anastomoses develop between both vitelline veins, one cephalad within the liver, one in the middle dorsal to the duodenum, and one caudad in front of the duodenum. This results in the formation of two rings. Subsequently the right half of the upper ring and the left half of the lower ring, together with the rest of the vitelline veins, regress, and the resulting single S-shaped vessel becomes the portal vein, which connects with the splenic and superior mesenteric veins. The proximal portion of the left vitelline vein above the hepatic plexus also atrophies, and the right vein, now draining the entire liver, develops into the hepatic veins.

In embryos 5.0 mm in length, the umbilical veins connect with the hepatic plexus and then the entire right umbilical vein and the portion of the left umbilical vein proximal to the connection with the liver atrophy. The rest of the left umbilical vein brings its oxygenated blood to the liver, through which it reaches the systemic circulation. At about 6.0 mm, a larger venous trunk develops in the sinusoidal system, shunting blood directly from the umbilical vein into the inferior vena cava. It subsequently separates from the sinusoids and becomes the ductus venosus Arantii, which carries about half the placental oxygenated blood to the heart. This duct is absent in many animals, and its basic function is not established [565]. The arterial blood supply of the liver probably originates from the arteries supporting the septum transversum. The vascular pattern is completed in embryos 24 mm in length or eight weeks of age.

Fetal Circulation. The fetus receives oxygenated blood only through the umbilical vein from the placenta (Fig. 67). Some of the blood passes first through the liver to the hepatic vein. Most of it goes through the ductus venosus directly into the inferior vena cava. The blood which the inferior vena cava brings to the heart is more saturated with oxygen than any other blood in the fetus, despite the admixture of venous blood from the splanchnic and caudal areas. In the right atrium most of the flow from the inferior vena cava is diverted by the septum secundum through the open foramen ovale into the left heart, which

then supplies the heart and upper part of the fetus, especially the brain, with relatively oxygen-rich blood. The blood from the superior vena cava passes through the right ventricle into the pulmonary artery and then largely through the ductus arteriosus into the descending aorta, since the thick-walled intrapulmonary branches offer great resistance to the pulmonary flow.

At birth a sphincter at the origin of the ductus venosus closes [150], possibly as a result of nervous stimuli, to prevent the loss of blood from the placental vein. The ductus venosus atrophies and becomes the ligamentum venosum, while the vestigial remnant of the umbilical vein is transformed into the ligamentum teres in the falciform ligament. The shunt through the foramen ovale stops immediately after birth because of the pressure changes in the heart. The foramen closes gradually by fusion of the atrial septums; closure is complete in 75 per cent of persons. The ductus arteriosus begins to obliterate, and after 3 months of life it usually is completely closed. The pulmonary arterial branches become thin walled at birth.

Later Fetal Development. The further maturation of the liver is characterized by a gradual thinning of the plates, originally several cells thick (Fig. 66), to one-cell-thick plates by the time gestation is complete. The early embryonic liver seems to have an extensive extracellular phase in relation to the cellular or cytoplasmic phase [860]. This becomes reversed as gestation continues [1031].

In embryos larger than 23 mm in length, lobules begin to develop, and at that time the bile ducts run parallel to the portal vein branches. The lobulation of the liver depends greatly on the original distribution of the veins.

The liver is at its largest relative size after the ninth week of gestation, and at this time the right and left lobes can be easily recognized. The quadrate and caudate lobes become visible subsequently. The liver bulges downward from the diaphragm, with which it remains in contact only in the base area, and the peritoneal reflexions of the coronary ligaments develop. The right lobe receives mainly portal vein blood, while the left lobe receives the oxygen-rich blood of the umbilical vein; thus the left lobe is relatively larger in the fetus. After birth the left lobe becomes continuously smaller, supposedly also because of the unfavorable angulation of the left branch of the portal vein.

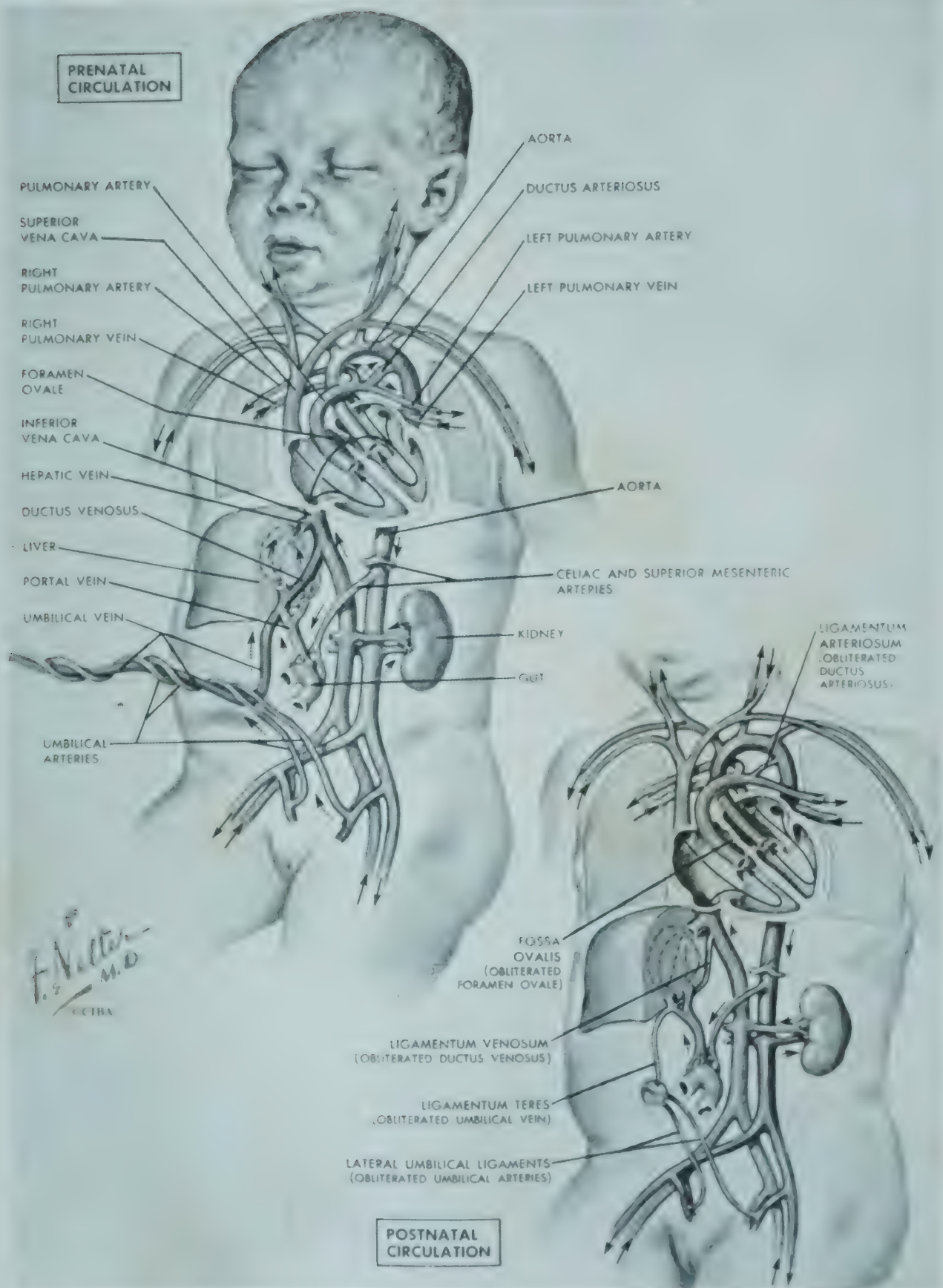


FIG. 67 Fetal portal circulation. (Copyright © The Ciba Collection of Medical Illustrations, Frank H. Netter, M.D.)

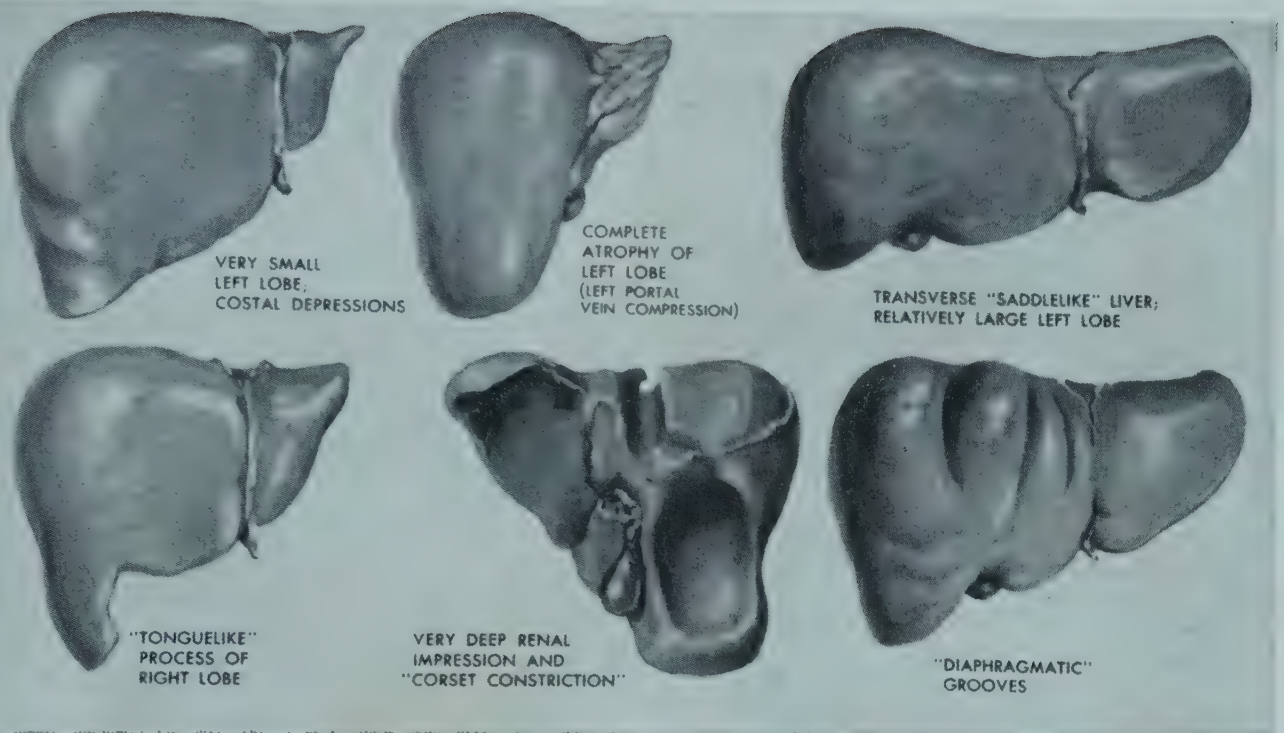


FIG. 68 Variations in the shape of the liver. (Copyright © The Ciba Collection of Medical Illustrations, Frank H. Netter, M.D.)

Postnatal Development. During the period of postuterine growth, the liver weight remains in constant proportion to the body weight [3071]. The postnatal development of lobules and parallel cytologic changes have been studied mainly in the pig, rat [2140], and mouse [752]. New lobules form and compound lobules develop by sprouting of central veins and ingrowth of portal tracts between the hepatic cells around the newly subdivided veins. The hepatic cells multiply and enlarge in size, and binucleated cells are found. This process of growth is especially prominent in the intermediate zone, which is physiologically less active [2140]. Glycogen appears in the guinea pig liver near the end of gestation, when synthesizing enzymes appear [2429]. It is fairly stable, since glucose-6-phosphatase, the splitting enzyme, is absent [2428]. Glycogen accumulation increases, whereas fat seems to decrease in the postnatal period [752].

Species Differences. The metabolic importance of the liver is expressed by the fact that in carnivores it is relatively much larger than in herbivores. In omnivores, such as man, it has an intermediate size. It is relatively larger in small animals and in the embryonal state, because of the higher metabolic rate. The great variations in the embryonic development in different species [907] are remarkable, since the end product is similar in all more highly developed animals.

In lower chordates it may remain only as a circular diverticulum. In most vertebrates the hepatic-cell plates are two or more cells thick.

MALFORMATIONS AND MALPOSITIONS

Liver

Most congenital abnormalities of form or position of the liver, extensively discussed by Hanser [1379], have little clinical significance. The borderline between abnormalities and simple variations is often hard to draw.

Malformations. ABNORMAL LOBULATION. An abnormal lobulation of the entire liver is produced by various irregularly arranged incisuras, primarily of the anterior edge and in the right lobe (Fig. 68). Whether the alteration is congenital or developed during life is often impossible to decide. Lobulation results from traumatic or inflammatory processes such as the syphilitic *hepar lobatum* (see *Hepatic Gummas* and *Hepar Lobatum*, under *Hepatic Syphilis*, Chap. 54), from pressure exerted upon the liver tissue, including in utero, or from vascular interference, with subsequent degenerative changes.

POLYPS. Pedunculated polyps of normal liver tissue, up to the size of hazel nuts, connected with the convexity of the liver are transitional

stages toward completely separated accessory livers in the omentum, the hepatic ligament, or fundus of the gallbladder. On a histologic basis, these rare pedunculated masses show regenerative hyperplasia, possibly from areas of preceding hepatic-cell degeneration, rather than neoplasia [205]. They have to be differentiated from pedunculated hamartomas (see Solid or Mixed Hamartomas, Chap. 57).

HYPOPLASIA OF LOBES. Either the left lobe or, less frequently, the right lobe may be hypoplastic or absent, with compensatory hypertrophy of the other lobe [84]. Complete absence of the liver has been reported only in the greatly deformed *acardius amorphus*. In *situs inversus* the liver is on the left side, or it may be only partially displaced.

INDENTATIONS. Indentations on the surfaces of the liver develop mainly during life. They are of several types.

Costal Arch Grooves. A broad, almost horizontal groove through the convexity of both lobes results from compression by the costal arch from tight clothing, especially corsets. In the furrow, a fibrous thickening of the capsule may be noted, with atrophy of the hepatic parenchyma below. In previous years, extremes of this lesion, with bizarre deformities, were found, and the deformed liver felt like a mobile kidney on palpation. The concurrent deformity of the gallbladder resulted in stone formation or jaundice caused by kinking of the extrahepatic biliary duct. Although the bizarre malformations of the corset liver are disappearing, one type of tongue-like extension of the right lobe, Riedel's lobe, is still found. This is a downward extension of the anterior edge of the right lobe around and to the right of the fundus of the gallbladder (Fig. 68). The extreme form, in which a fibrous bridge containing bile ducts connects Riedel's lobe with the rest of the liver, is rarely noted. Lesser degrees of this deformity create differential diagnostic problems on palpation, especially since this lobe is harder than the rest of the liver. The distortion may also alter gallbladder function. This association resulted in the assumption that inflammatory gallbladder diseases cause this extension of the right lobe.

Rib Impressions. In very large livers parallel arcuate impressions are produced on the right aspect by the individual ribs. They are also seen in very old people, because of the rigid thoracic cage, and in kyphoscoliosis.

Sagittal Furrows. Sagittal furrows on the convex surface, usually parallel and several in number, result from impressions of the posterior insertions of the diaphragm. In these grooves the capsule is not thickened and the hepatic tissue is not atrophic, suggesting an effect of molding only.

Congenital Diaphragmatic Herniation. Partial eventration of the right diaphragm may result in a focal prolapse of the liver, making it appear as an intrathoracic mass. It has characteristic radiologic features [3431].

Malpositions. The liver may be displaced upward or medially through diaphragmatic or umbilical hernias. It may be displaced downward by tumors, pleural effusions, or emphysema. This downward displacement produces the wrong impression of an enlarged liver. Downward displacement may also be due to relaxation of the suspending falciform and coronary ligaments (hepatoptosis). Hepatoptosis, or visceroptosis, very occasionally causes symptoms primarily owing to portal stasis. Examination in the supine and upright positions usually clarifies the condition. It is found more often in women than in men and has been associated with abnormal relaxation of the abdominal muscles and of the intraabdominal connective tissue.

Biliary System

Congenital abnormalities of the gallbladder and extrahepatic bile ducts are of major surgical significance and are discussed in detail in surgical texts and monographs [1297, 1379, 2839, 3477]. Here they are only briefly reviewed.

Intrahepatic Bile Ducts. ATRESIA. Atresia of the intrahepatic, or interlobular, bile ducts is usually associated with anomalies in the extrahepatic bile duct system [25, 1852]. Although the bile ducts apparently never develop in these cases, the patients may survive up to 10 years [2156], possibly because of lymphatic drainage of bile. Xanthomas of the skin with high levels of serum phospholipid, cholesterol, and total lipid are noted. At autopsy a form of biliary cirrhosis, congenital acholangic biliary cirrhosis, is found [583].

CYSTS. Partial atresias, with disturbed differentiation, segmentation, and dilatation of the intra-lobular bile ducts in the presence of normal differentiation of the other hepatic elements, result in hamartomatous abnormalities of the bile ducts, including formation of Meyenberg complexes and cysts (see Bile Duct Hamartomas, Chap. 57).

Extrahepatic Bile Ducts. ATRESIA. Congenital atresias of the bile ducts and the atresias of the intestinal tract are probably of similar developmental origin. Originally various inflammatory factors, such as congenital syphilis or fetal cholangitis, were considered as etiologic possibilities. The extrahepatic ductal system arises as a hollow tube. In certain stages of its development it becomes temporarily solid, at least in places. Persistence of the solid stage, with disappearance of the epithelium and subsequent hypoplasia of the mesenchymal covering in the area, accounts for all stages between hypoplasia with very narrow lumens, atresias, and only fine fibrous cords without epithelium. These cordlike structures may be so indistinct that complete aplasia is apparent, although it never actually exists. The atresia may occur at the lower end of the common duct, preventing its junction with the duodenum, or at the junction of the common and cystic ducts. Multiple atretic areas also occur, and no recognizable ducts or remnants may be found between the hilus of the liver and the duodenum. The duct proximal to the atresia is rarely dilated. This is also true of the gallbladder, which is sometimes completely absent. Extrahepatic atresia is sometimes associated with atresia or absence of the small bile ducts throughout the liver [25, 1852, 3477]. Congenital atresia leads to chronic biliary hepatitis with secondary biliary cirrhosis [865], sometimes associated with splenomegaly and even to a xanthomatous stage [25]. Jaundice usually develops after the first or second week of life, since the liver produces little bile before birth or in the early neonatal period. The jaundice increases persistently, and the feces are permanently acholic, initially associated with low levels of serum bilirubin. In late stages, desquamation of intestinal epithelium may produce some bile staining of the feces. The urine remains dark but free of urobilinogen. Surprisingly little anemia develops [1297]. Children with congenital atresia gain relatively little weight but live for eight months on the average; survival until three years of age has been seen. They succumb to cholemia late, but are exposed to disturbances of intestinal absorption, particularly of lipids and vitamins A and K, so that morphologic manifestations of vitamin A deficiency [58], hypoprothrombinemia, and osteoporosis are noted. Death results from intercurrent infections or sometimes from gastrointestinal hemorrhage. Exploratory surgery and cholangiography should be performed in every

instance after 4 months, and if a patent duct is found, it should be anastomosed to the gastrointestinal tract. In some instances extensive procedures such as hepatojejunostomy may offer some relief [2729]. Anatomic considerations favorable for surgical anastomoses are found in only slightly more than 20 per cent of infants with involvement of the common or hepatic ducts [1297]. Atresia of the cystic duct is innocuous, producing only hydrops of the gallbladder.

"INSPISSATED BILE SYNDROME." Persistent or fluctuating jaundice in the first 3 months of life has been explained as the result of plugs in the extrahepatic bile ducts. The jaundice is said to fluctuate and supposedly decreases on irrigation of the ducts or after hydrocholeretic therapy [1297]. The differentiation from congenital atresia of the bile duct may be clinically difficult. At operation, the biliary ducts are of normal width, but they contain plugs, or inspissated mucinous bile. Some investigators consider this lesion to be caused by formation of thick mucous bile as a congenital defect (mucoviscidosis). Today most observers believe that this condition is the result of inspissated bile because of cholestasis in neonatal hepatitis, probably of viral etiology, or in hemolysis. The bile plugs probably are the result rather than the cause of the bile stasis.

ABERRANT DUCTS. Variations occur in the course of the cystic, hepatic, and common bile ducts, some of which are surgically important (Figs. 41, 69, lower left). Accessory bile ducts are aberrant segmental ducts [1437]; they may assume the function of the main hepatic duct if it is atretic [2466] (see Cystic Duct, under Gallbladder, Chap. 15).

CHOLEDOCHAL CYSTS. Idiopathic choledochal cysts sometimes appear as retroperitoneal tumors and reach the size of a man's head [3531]. They displace the stomach to the left and the duodenum downward, and they may produce biliary obstruction with jaundice. Moderate diffuse dilatation without cyst formation results from any obstruction of the duct. In contrast, cystic dilatation is presumably either the result of congenital circumscribed weakness of the wall, with hypoplasia of the muscular layer, or the result of irregular epithelial proliferations while the common duct is still solid [3017]. Kinking or a valvelike occlusion at the papilla of Vater may enlarge the cystic dilatation and obstruct the bile flow. The enlargement can occur later in childhood, primarily in females [425, 3917]. Jaundice is found



FIG. 69 Cholecystograms. *Upper left.* Bifid gallbladder. *Upper right.* Gallbladder in lateral aspect of right lower quadrant. *Lower right.* Gallbladder in medial aspect of right lower quadrant overlying the sacroiliac joint. *Lower left.* Gross specimen showing absence of cystic duct and anomalous entry of hepatic duct (dark hole in center) directly into a dilated and inflamed gallbladder and common duct. (All parts except lower left, courtesy of Dr. Lillian Donaldson and Dr. Geza G. Kopstein.)

in 60 per cent of the cases, but obstruction is not complete and biliary cirrhosis need not occur. The lesion may involve only the junction of the cystic and hepatic ducts or the entire common and hepatic ducts. In rare instances it even extends to the pancreatic ducts. The gallbladder may be of normal size even if the cystic duct is involved. Stones and carcinomas have been reported in these cysts, as well as calcification of

the cyst wall. Acute infections may lead to rupture or at least to acute pain with peritoneal irritation. Cystic changes have been found in the extrahepatic bile ducts of mice after transplantation of functional pituitary tumors [1111]. Small diverticula, sometimes containing stones, have been found in the terminal portion of the common duct and have been thought to produce pancreatitis [3201].

Gallbladder. MALFORMATIONS. Complete absence of the gallbladder is normal in some animals, but it is rare in man. This condition may or may not be associated with atresia of the bile ducts. Anomalies of the gallbladder have been noted in 2.8 per cent of cholecystograms; symptoms of gallbladder disease were present in 70 per cent of this group, while stones were found in 14 per cent [2041]. The gallbladder may be hypoplastic, a condition which is occasionally not easily differentiated from inflammatory scarring, especially in adults. Absence or hypoplasia of the gallbladder is not necessarily associated with compensatory dilatation of the common duct. The gallbladder may be completely reduplicated, with or without duplication of the cystic duct or cystic artery. One of a pair is often small and accessory. Partial subdivisions such as bifid gallbladders (Fig. 69, upper left) or diverticula are more common [2839, 3550]. Bilobed gallbladders are often seen in cats [365, 366, 367]. Diverticula are found in any part of the gallbladder. Their differentiation from inflammatory lesions is often difficult, since pseudo-diverticula result either from deep extension of the surface epithelium or from general weakening of the wall. Diverticula are important as a possible site of perforation. The frequently found phrygian, or stocking, cap of the gallbladder is an indentation in the fundus either with the serosa participating, which makes the lesion obvious without opening the organ, or with the serosa covering the fold. The phrygian cap results from faulty embryonal development of the gallbladder in relation to its liver bed, and it seems to have little influence on the function of the organ [365, 366, 367], although it may sometimes lead to pain and distress not relieved by amyl nitrite. An indentation in the middle of the gallbladder produces the infrequently found "hourglass" gallbladder. Rarely, heterotopic gastric mucosa is found in the gallbladder wall [2144].

MALPOSITIONS. Malpositions include left-sided [952], median, transverse, and ptotic positions of the gallbladder (Fig. 69, upper right and lower right). Abnormal relations to the liver range between two extremes. In one, the gallbladder is completely surrounded by serosa in the form of a mesentery and is not attached to the liver—a floating gallbladder. This abnormality may develop in elderly persons, especially women who have lost weight. A twist of the attachment results in hemorrhagic infarction. The other extreme is the complete inclusion of the gallbladder within the

liver, a continuation of the embryonal relationship of little clinical significance, except for interference with the contractibility of the gallbladder. Bands connecting the gallbladder with the colon have been called "cystocolic ligaments." This peritoneal duplication is a continuation of the lesser omentum and is said to produce biliary stasis and stones. Differentiation from inflammatory adhesions may be difficult.

AGONAL AND POSTMORTAL CHANGES

The study of liver biopsy specimens permits the recognition of changes which occur after death or within the agonal period and which, therefore, differentiate the autopsy specimen from the biopsy specimen. The changes depend not only upon the duration of the agonal period and the time interval between death and fixation but also upon the cause of death [2348, 2625]. Extreme differences between needle aspiration specimens taken immediately after death and autopsy specimens obtained 18 hours later have been noted [3404] (Fig. 70).

PROTEOLYSIS. This depends more upon the basic disease than do the other processes. Proteolysis is most severe in liver disease and infections. It causes structural alterations of hepatic nuclei and cytoplasm that are particularly apparent with special cytologic methods, making the use of many techniques, such as mitochondrial or enzyme stains, impossible in autopsy material. Initially, nuclei and cytoplasm stain less intensely, and karyorrhexis develops. With prolonged autolysis the cytoplasm stains more deeply basophilic [2348]. Advancing autolysis has been associated with alterations of the isoelectric point of the cytoplasm. The Golgi apparatus disappears, and the nuclear staining, as well as the cell borders, becomes less distinct. Finally, a homogeneous acidophilic mass results. The proteolytic changes are similar to intravital autolytic changes as seen in traumatic necrosis or transplantation.

GLYCOGENOLYSIS. Wasting diseases or a prolonged agonal period tend to use up the glycogen stores of the liver without replacement. The loss of glycogen from the cytoplasm is obvious even in sections stained with hematoxylin-eosin, in that the hepatic-cell plates are thinner and the cells smaller, and the cytoplasm stains darker and is more homogeneous than in glycogen-rich hepatic cells. This difference is recognized without glycogen stains and is the most obvious distinction

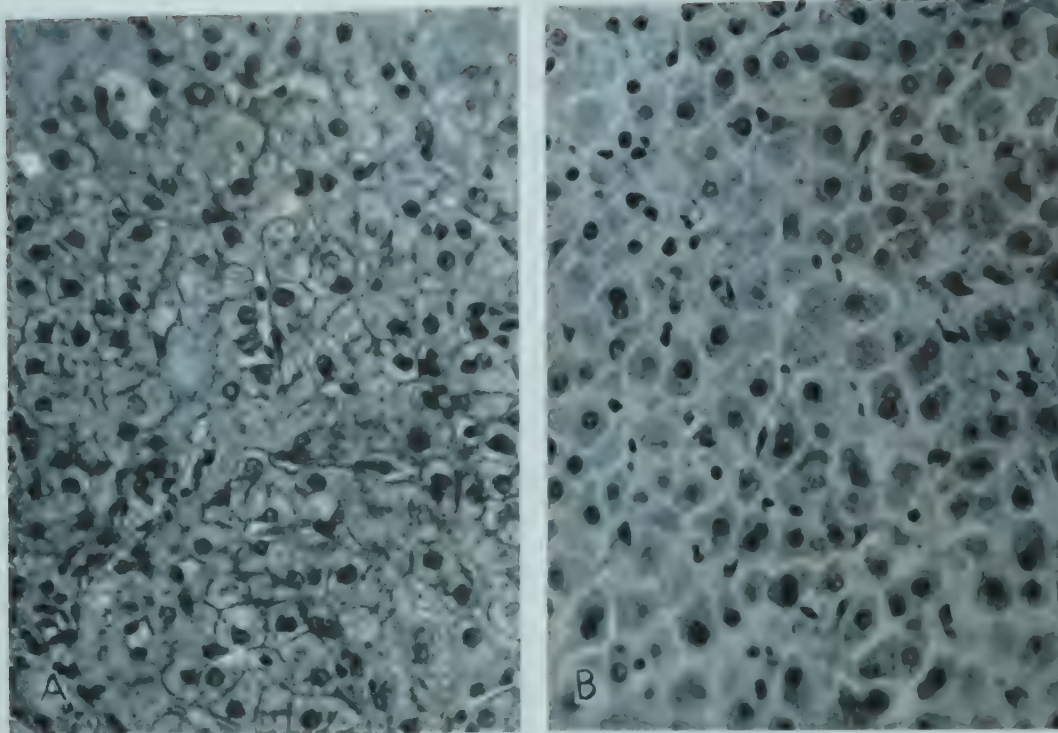


FIG. 70 A. Biopsy specimen of liver taken 13 hours before death in a patient with mild toxic hepatitis. The structure of the hepatic-cell plates is only slightly distorted, and the perisinusoidal spaces are almost completely closed. The hepatic cells appear to be rich in glycogen. B. Autopsy specimen obtained in same patient. The hepatic cells are dissociated. The isolated cells appear to be depleted of glycogen, and the tissue spaces between the cells and sinusoids are open. (Popper, H.: *Arch.Path.* 46:132, 1948.)

between the usual biopsy and autopsy specimens. Only following sudden death does the cytoplasm appear finely and irregularly vacuolated in routinely stained autopsy specimens, in contrast to biopsy specimens in which this is usually the case. Absence of glycogen in biopsy specimens as a result of malnutrition or disease has far more diagnostic significance than the same finding in autopsy specimens. The glycogen staining of the cytoplasm has been ascribed medicolegal significance in the recognition of sudden death. Chemical analysis has shown that the greatest part of the glycogen is lost in the first hour after death, but chemical and histologic changes may not be parallel [2352].

ALTERATION OF LIPIDS. Lipids appear resistant to postmortal processes except in far-advanced autolysis, where more fat than normal becomes stainable. The vitamin A fluorescence becomes reduced and abnormal within 24 hours [2625].

WIDENING OF THE INTERSTITIAL SPACES. In autopsy specimens, the tissue spaces of Disse are usually wide open and filled with albuminoid debris, while in most biopsy specimens and in specimens obtained from persons dying instantly, these spaces are completely obliterated [2625]

(Fig. 32) (see Tissue Spaces, under Lymphatic Vessels and Tissue Spaces, Chap. 19). This also may have medicolegal significance, in that obliterated tissue spaces in autopsy specimens speak for instantaneous death. In general, the tissue spaces widen before the glycogen begins to disappear. Open tissue spaces in liver biopsy specimens are abnormal. In most experimental animals the expansion of the tissue spaces is less and does not develop during the agonal period.

VARIATIONS IN BLOOD CONTENT. Needle biopsy specimens, as a rule, contain little blood. It either has oozed out or has been squeezed out of the specimen before and during fixation. In the larger surgical biopsy specimen, the blood content is less reduced. Swelling of the glycogen-laden hepatic cells is probably an additional factor in reducing the blood content. In autopsy specimens, the terminal heart failure increases the blood content of the sinusoids. Neither autopsy nor biopsy specimens mirror the blood content of the sinusoids in life.

CENTRAL NECROSIS. Some centrolobular necrosis develops during the agonal period and is a result of terminal heart failure, and central areas of necrosis are noted in autopsy specimens which

were not seen in biopsy specimens obtained shortly before death [2625; 3541]. With preceding severe liver damage, the lobule may become disrupted [3404].

BACTERIAL INVASION. The anaerobic gas-producing bacteria alter the appearance of the liver after death. Their presence results in the formation of a foamy, soft liver. Bacteria may readily be demonstrated, especially in vessels.

INFLAMMATORY REACTION. Biopsy specimens from patients with conditions such as viral hepatitis with spotty necrosis, nonspecific reactive hepa-

titis, intrahepatic cholestasis, or cholangiolitis, and rapidly advancing (florid) cirrhosis contain inflammatory cells, either neutrophils or round cells, around necrotic or necrobiotic hepatic cells. In autopsy specimens from patients with the same conditions such accumulations appear to be less conspicuous. Sometimes the difference in inflammatory exudate between specimens obtained at autopsy and biopsy from the same patient is surprising, even though the interval between the taking of the two specimens may be only a few days.

PART II

*Pathologic Phenomena
in the Hepatobiliary System*

Jaundice means the presence of excess bile pigments in tissues and in serum. It is the presenting sign in a number of hepatic and nonhepatic diseases. The classification of the types of jaundice should depend upon their pathogenesis. The mechanism of hyperbilirubinemia, the physico-chemical state of the bile pigment, the relation between serum and tissue concentrations of pigment, and the relation between the levels of bile pigment and other biliary constituents in the blood are less certain today than they were 20 years ago, despite intensive efforts by biochemists, physiologists, pathologists, and clinicians. A clarification of the complex problem may result either from improved knowledge of the biochemistry of the bile pigments, possibly with the aid of isotopic tracer methods, or from the visualization of the pathway of the bile pigment through the liver by modern histophysical and histochemical methods. This problem is laden with many arguments, well illustrated in earlier texts [943, 1996, 2784, 2804] as well as in many monographs. Some of the most widely quoted classifications and theories will be briefly reviewed before a simple, noncommittal classification will be presented which allows for changes and amplifications.

CLASSIFICATION OF JAUNDICE ACCORDING TO EXISTING THEORIES

Theories of the Nineteenth and Early Twentieth Centuries. Jaundice has been explained as being a leakage of bile pigment into the blood, a "diapedesis," from the site of its formation, the hepatic cells (paracholie). A series of objections to this explanation arose. After ligation of the common bile duct, the bile pigment concentration rose in the thoracic duct before it did in the serum

[943, 1220]. This indicated leakage into the tissue spaces and lymphatic vessels rather than into the blood. The assumption that bile pigment is formed in the hepatic cell appeared untenable. Virchow recognized that bile pigment, hematoidin, can be formed outside the liver. When arsine and toluylenediamine were found to produce jaundice, the question arose whether they could do so in hepatectomized animals. In hepatectomized geese, Naunyn was unable to produce jaundice with these poisons; this seemed to point to the essential role of the hepatic cells in bile pigment formation. Subsequently, these poisons were recognized as hemolytic agents, and geese were found to differ from other animals in that their livers contain the entire reticuloendothelial system, which is responsible for the steps in the breakdown of hemoglobin to bilirubin. Eventually in the twentieth century, under the influence of Aschoff [108] and van den Bergh [3408], the formation of bilirubin was definitely localized in the reticuloendothelial system, including the Kupffer cells, and the hepatic cells were assumed merely to transform the form giving the indirect van den Bergh reaction to the direct- or prompt-reacting form (see Bilirubin, in Chap. 11).

Eppinger's Theory. A morphologic method for demonstration of the bile canaliculi and the knowledge that bile pigment is formed in the reticuloendothelial cells and only secreted by the hepatic cells made possible a better understanding of bile pigment metabolism.

In mechanical biliary obstruction, Eppinger demonstrated dilatation of the bile canaliculi histologically [943]. Ramifications of these dilated canaliculi extended between the hepatic cells, and when they became larger they reached to the interstitial spaces. These ramifications were thought

to rupture and form funnel-shaped communications, through which bile seeps into the interstitial tissue and from there ultimately into the blood. In early stages of obstructive jaundice this rupture was not visible, and mere diffusion under increased pressure was assumed. In parenchymatous jaundice, hepatocellular degeneration, or necrosis, supposedly breaks the continuity of the hepatic-cell plates, and bile escapes through funnel-shaped ruptures of the bile canaliculi into the interstitial tissue. A modification of this original concept of Eppinger localized the primary rupture or abnormal diffusion into the ductules, which Aschoff had called the "heel of Achilles" of the biliary system because of their thin wall.

Moreover, edema, lymphorrhea, or even fibrosis was assumed to produce bile stasis in the ductules in diseases characterized by injury of the hepatic parenchyma.

In jaundice from increased blood destruction, inability of the liver to remove all the bilirubin formed by the reticuloendothelial system was considered the primary cause. A mechanical effect was also assumed, since Eppinger found inspissated bile, bile plugs, or thrombi in the dilated canaliculi [943]. He felt that these thrombi formed because of the presence of protein in the viscid pigment-rich bile in hemolytic conditions.

Eppinger modified the statement of Naunyn and Minkowski from "icterus could not develop without a liver" to "icterus could not develop without faulty excretion of bile" [943].

McNee's Classification. McNee presented a theory and classification of jaundice which introduced into the English medical literature a concept similar to Eppinger's. He differentiated hemolytic, obstructive, and hepatocellular (originally called toxic and infectious) jaundice [2804]. He emphasized the combination of the three processes, viz.: (1) liver damage in biliary obstruction; (2) hepatic functional alteration in hemolytic jaundice; (3) intrahepatic obstructive phenomena in hepatic damage caused by edema or cellular debris.

Theory of Rich. Rich attached relatively little significance to disturbance of the excretory function of the hepatic cells in the pathogenesis of most forms of jaundice [2748]. Three basic causes of jaundice in the absence of mechanical biliary obstruction were theoretically considered: (1) inability of the hepatic parenchyma to remove bilirubin from the blood, implying an elevated hepatic threshold for biliary secretion; (2) supply of bilirubin to the liver in excess of its ability to

excrete it; (3) faulty excretion of bilirubin by the liver even at normal serum-bilirubin levels. Rich stressed the great reserve of the healthy liver in excreting bilirubin [2748] and referred to animal experiments in which ligation of several branches of the hepatic duct did not lead to jaundice. Clinical jaundice was divided into retention jaundice, caused by overproduction of bilirubin by the reticuloendothelial system associated with subnormal function of the liver which is unable to accept all of it, and regurgitation jaundice, produced by necrosis of hepatic cells, obstruction of bile ducts, or some unknown factors [2748]. Anoxemia, either from low hemoglobin levels or from subnormal oxygen saturation, was considered to be an important factor in depressing the function of the hepatic cell. Therefore, anemia, pneumonia, or heart failure produce jaundice, especially if associated with increased blood destruction in the congested liver or with a pulmonary infarct. Immaturity of hepatic cells, as well as fever, was supposed to depress hepatic function. Rich emphasized that the indirect van den Bergh reaction, the absence of bilirubin and of bile acids from the urine, and the increased fecal pigment permitted simple clinical differentiation of retention from regurgitation. The separation of retention and regurgitation jaundice represented great progress. In clinical diagnosis, however, it mainly confirmed the separation of hemolytic, or overproduction, jaundice from the other forms (obstructive and parenchymatous jaundice, as described by Eppinger and McNee). In obstructive and parenchymatous jaundice, both retention and regurgitation of bile pigments may occur, depending upon the extent and stage of the lesion.

Pavel's Theory. Pavel assumed that bilirubin is transformed into the prompt-reacting form in the Kupffer cells and is passed, with filtered biliary fluid, directly into the bile canaliculi without passing through the hepatic cells [2544]. He demonstrated pseudopodial processes of the Kupffer cells extending between the hepatic cells to the bile canaliculi. The hepatic cells supposedly reabsorb water from the filtrate in the bile canaliculi but do not participate in the excretion of bile pigment. The balance between filtration and reabsorption is disturbed in the various types of jaundice. He recognized the following factors: (1) hemolysis; (2) mechanical obstruction to bile flow; (3) functional obstruction primarily caused by spasm of the sphincter of Oddi; (4) hepatitis in which prompt-reacting bilirubin is diverted from the

Kupffer cells into the blood stream owing to hepatic-cell damage; (5) combination of these factors, such as exists in viral hepatitis.

With's Theory. With rejected the division of bilirubin into prompt-reacting and indirect-reacting forms and emphasized the possibility that blood pigment may be broken down to pigments other than bilirubin [3638]. He differentiated three pure types of jaundice: (1) production jaundice, from excessive production of bilirubin; (2) retention jaundice, caused by reduced excretory capacity of the liver, which would occur only if the functional hepatic tissue is less than 5 per cent of normal; (3) lymphogenous jaundice, either from rupture of bile canaliculi or from an abnormal secretion of bile from the blood through the hepatic cells into the lymph capillaries. The presence of jaundice without rupture of bile canaliculi was explained as being caused by a special secretory mechanism which occurs as the pressure rises in the bile passages. The clinical types of jaundice were described as combinations of the three basic forms. Parenchymatous, or hepatocellular, jaundice was considered a combination of decreased ability to remove bilirubin (retention) and lymphogenous regurgitation with disturbed continuity of the hepatic cells by hepatic necrosis. Obstructive jaundice was thought to result from retention combined with lymphogenous regurgitation, while production jaundice, usually associated with retention, was said to account for hemolytic jaundice. The familial nonhemolytic jaundice was considered to be an inborn error of the metabolism of bilirubin resulting in excessive formation. Icterus neonatorum was listed as a result of increased production following rapid blood destruction [2328] and retention owing to bypassing of blood before the closure of the ductus venosus [3638].

Other Theories. Many modifications of the major theories and classifications have been presented [3081, 3547]. One author stressed the importance of disturbances in the centrolobular zone, where the accelerated blood flow supposedly interferes with bilirubin excretion, resulting in hyperbilirubinemia [926]. The same author also differentiated between a permanent, or adynamic, elevation of the excretion threshold of bilirubin, such as is found in familial jaundice, pernicious anemia, and in horses physiologically, and a transient, or dynamic, elevation caused by temporary inactivity of hepatic lobules, such as is supposedly present in lobar pneumonia.

Watson and Hoffbauer have differentiated in the parenchymal group of jaundice, primary involvement of the parenchymal cells, or hepatocellular jaundice, from primary involvement of the ductules, hepatocanalicular or cholangiolitic jaundice, and have correlated each phenomenon with clinical and laboratory findings [853, 3510]. In the cholangiolitic form, regurgitation was said to occur through morphologically intact ductules because of abnormal permeability.

Another widely used classification of jaundice is prehepatic jaundice for the hemolytic form, hepatic jaundice for the parenchymal form, and posthepatic jaundice for extrahepatic biliary obstruction.

CRITICISM OF EXISTING THEORIES

The mere existence of a large number of theories indicates the lack of a satisfactory one. Several points of criticism can be leveled against all the existing theories.

The van den Bergh-Aschoff Concept. Most theories accept the van den Bergh-Aschoff concept that prompt-reacting bilirubin has passed through the hepatic cells, while indirect-reacting bilirubin has not. In the chapter on bile pigment metabolism, the validity of this assumption and of the assumption of chemical differences in bilirubins was discussed (see Bilirubin, Chap. 11). Earlier experiments indicated that the level of indirect-reacting bilirubin increases first in obstructive jaundice, but modern studies indicate that prompt-reacting bilirubin rises first, in the thoracic duct lymph, supporting the idea that it is regurgitated [1220]. In both extrahepatic biliary obstruction and parenchymatous hepatic disease, the relation between the two forms of bilirubin is similar and depends on the bilirubin level [2906, 3708], which fact is not in keeping with the idea that all prompt-reacting bilirubin is regurgitated. In both groups of diseases the ratio of prompt-reacting to total bilirubin is low under normal circumstances and increases with increasing hyperbilirubinemia, reaching a maximum of 50 to 60 per cent at levels of 5 to 10 mg per 100 ml serum bilirubin (Fig. 71). According to the van den Bergh-Aschoff theory, this would imply equal degrees of regurgitation in mechanical obstruction and parenchymal jaundice. It would also imply that even in severe necrotizing hepatic damage, the uptake of bilirubin by the hepatic cells and its transport into the bile canaliculi remain unchanged. This as-

sumption appears particularly untenable in severe hepatic necrosis, where few intact hepatic cells can be demonstrated, despite high concentrations of prompt-reacting bilirubin. Therefore, a retention of bilirubin or a block in the transmission of previously transformed bilirubin must be assumed.

Limits of the Hepatic Excretory Reserve. Earlier experiments indicated that the hepatic ducts draining 95 per cent of the liver have to be ligated before jaundice appears. Similarly, only transient hyperbilirubinemia develops after removal of a greater part of the liver [339]. The overproduction of bilirubin should therefore be excessive to produce jaundice. In this sense, hemolysis would cause jaundice only if it were very extensive or associated with liver damage. This assumption, which makes uncomplicated hemolytic jaundice unlikely, is not necessarily valid, in view of the results of the parenteral injection of bilirubin [1454]. Small amounts produce hyperbilirubinemia and visible jaundice, and the clearing ability of the liver appears to be less than

expected. Hemolytic, or retention, jaundice is possible, therefore, without associated hepatic insufficiency. The similarity to the behavior of injected alkaline phosphatase is remarkable (see Phosphatases, in Chap. 7).

Absence of Jaundice in Severe Hepatic Injury. In acute hepatic insufficiency confirmed by morphologic examination, jaundice may be entirely absent. Lack of time has been offered as an explanation of acute conditions, but the lack of jaundice in cirrhosis with hepatic failure has to be related to some process outside the hepatic cells. This process may possibly be altered blood flow or altered pathways of blood breakdown.

Morphologic Demonstration of Regurgitation. Eppinger [943] described discontinuity of hepatic-cell plates in parenchymal and obstructive jaundice, resulting in funnel-shaped communications between the bile canaliculi and tissue spaces (see Eppinger's Theory, above). For many years this morphologic picture was considered the basis of regurgitation of bile from the bile canaliculi.

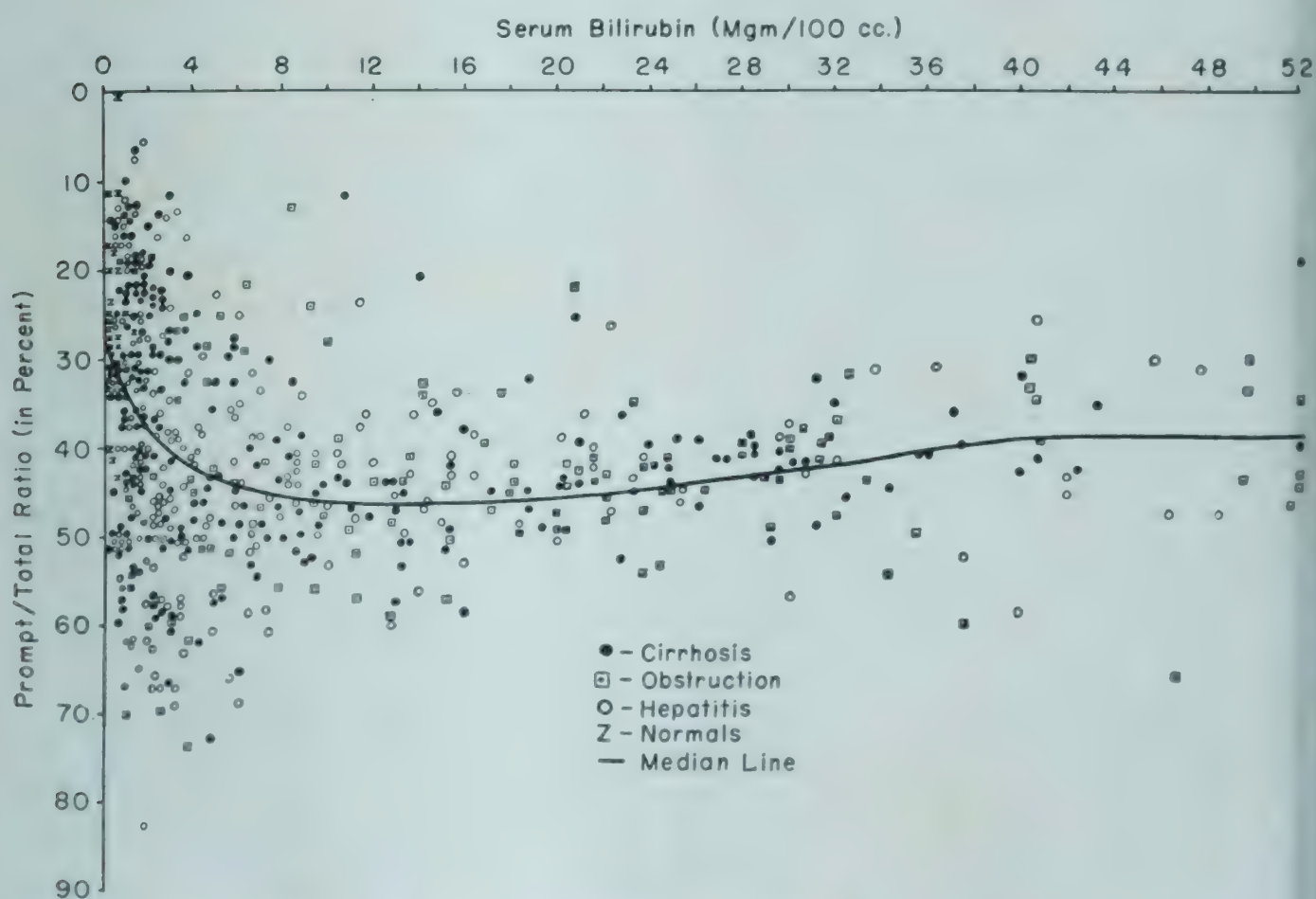


FIG. 71 Correlation between total serum bilirubin and the prompt-reacting total bilirubin ratios in normal persons and in patients with various hepatobiliary diseases. (Schaffner, F., Popper, H., and Steigmann, S.: *Am J M.Sc.* 219:307, 1950.)

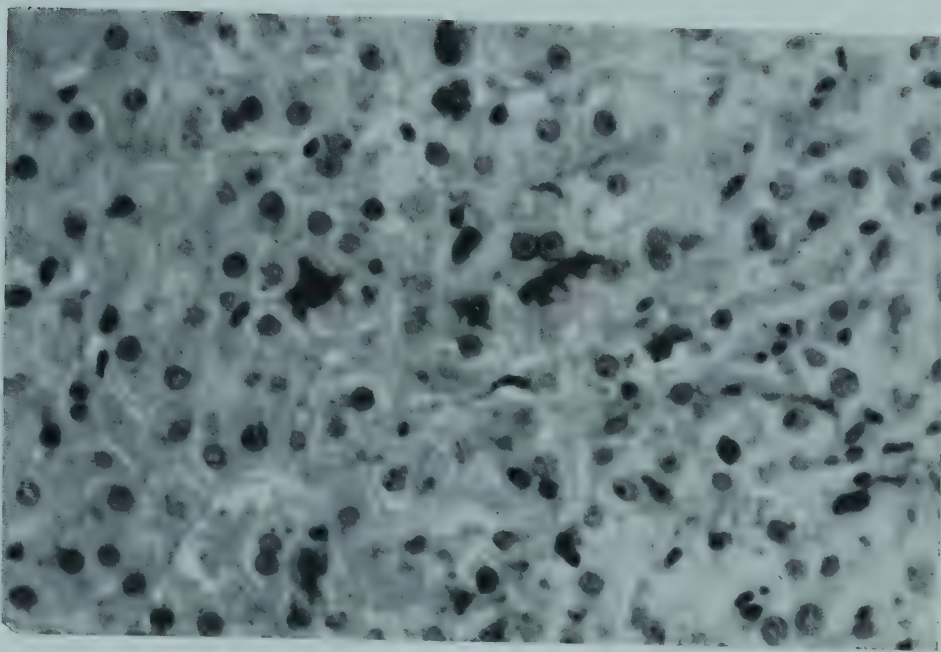


FIG. 72 Ramified-appearing bile casts in extrahepatic cholestasis. H&E ($\times 330$).

Eppinger's observations and those confirming them were based upon the study of autopsy material. As biopsy specimens became available, disruption of hepatic-cell plates was not necessarily apparent in them in either obstructive or parenchymal jaundice [2801, 2802]. Moreover, comparison of the same livers in biopsy and autopsy specimens shows that the hepatic-cell plates become discontinuous in the agonal or postmortal period [2625] (Fig. 70). This discontinuity therefore can not be considered the cause of regurgitation. Rupture of bile canaliculi is also unlikely, since these structures are more resistant in teasing preparations than the rest of the hepatic-cell plate. The fact that regurgitation can not be demonstrated morphologically at these levels does not completely exclude it; nevertheless, this fact remains the strongest argument against the theory. This negative finding lends support to the idea that regurgitation may take place elsewhere, perhaps from the ductules.

Bile Casts. In many forms of jaundice, ramified casts are found in dilated bile canaliculi (Fig. 72). Even in routine histologic sections they seem to consist of inspissated bile; they have been called bile thrombi, cylinders, or plugs [943]. Bile casts are found within bile canaliculi and are usually associated with the presence of pigment granules in the hepatic cells. Eppinger assumed that the bile casts develop as a result of abnormal mixing of bile and protein, "albuminochole," from cellular breakdown products [943]. Support for this idea is seen in the many bile casts in jaundice from toluylenediamine hemolysis, in which albuminochole was demonstrated. Similarly shaped

simple protein casts are seen without bile pigment in early stages of viral hepatitis [1872] or after exposure to lowered atmospheric pressures [55]. After the hepatic cells disintegrate, the bile casts are engulfed by Kupffer cells.

Bile casts were thought to be obstacles which produced jaundice, rather than results of jaundice. In this sense, any type of jaundice would be mechanical. Subsequently, many other explanations were offered for bile plugs, such as hepatic-cell damage, bile stasis, age, inflammation, and even avitaminosis [1903]. The explanation most acceptable at present is that bile thrombi result from bilirubin stagnating in lobular zones where the liver does not secrete bile [3541]. In most types of jaundice, centrilobular hepatic-cell damage is present, with apparent inability of these cells to secrete bile. The peripheral and intermediate bile-producing zones fill the empty central bile canaliculi with bile, which then stagnates and precipitates with protein. In severe hepatic injury, such as in viral hepatitis, bile casts are absent because of the lack of bile secretion and rupture of the bile canaliculi. They appear in the defervescent stage [1714]. Moreover, the facts that bile casts first appear in the central zone of the lobule in all types of jaundice and that they appear also on the periphery only in very protracted cases speak against a mechanical role of bile thrombi in the development of jaundice.

Hepatic-cell damage can not be the sole cause of the bile casts, because in jaundice without hepatic-cell damage, such as methyltestosterone jaundice, many bile casts are seen [3559]. In ob-

structive jaundice casts may persist after the disappearance of the jaundice.

Intrahepatic Biliary Obstruction (*Intrahepatic Cholestasis*)

Frequently the absence of bile from the intestine can be demonstrated in diseases in which extrahepatic obstruction can be excluded with certainty either at operation or at necropsy. While complete absence of bile is unusual, some decrease is common [3183] in many types of hepatitis with jaundice. Several possible causes for this phenomenon have to be considered.

Mechanical Interference with Bile Flow. OBSTRUCTION OF BILE CANALICULI. The possibilities that bile canaliculi can be mechanically obstructed by bile casts and by disruption of the hepatic-cell plates [945] have been discarded (see Bile Casts and Morphologic Demonstration of Regurgitation, above). The possibility of some compression of the bile canaliculi by swollen hepatic cells [1497] is supported by the observations that overloading of hepatic cells with glycogen causes hyperbilirubinemia and bilirubinuria, and that excess fat storage in the liver during ethionine intoxication leads to hyperbilirubinemia without other functional damage [1820]. At least a part of the bile drained from the lobule passes through bile canaliculi in the limiting plate before entering the ductules [907]. Therefore, the possibility exists that a disturbance in the limiting plate interferes with biliary drainage [2463]. The frequently found alterations of this plate, however, do not parallel the incidence or degree of jaundice.

OBSTRUCTION OF DUCTULES. Inflammatory edema or exudate on the border of the portal tracts may compress the ductules. Separation of the ductules from the bile canaliculi by exudate has also been suggested [3183, 3638], with reunion as a result of proliferation of the ductules. Bile flow, however, may be decreased or absent in the absence of any portal inflammation in biopsy specimens, and it may be present with severe inflammation in the portal tracts [1903, 3182]. Obstruction by microcalculi or by scarring seems to be far more significant. In many types of hepatitis, relatively large plugs appear to dilate the lumens of the ductules. The epithelium is stretched and sometimes missing, and microcalculi are in direct contact with the basement membrane (Fig. 83A, B). In some forms of chronic hepatitis or cirrhosis, the perilobular ductules are kinked or even destroyed [2155, 2156].

OBSTRUCTION OF THE SMALLEST BILE DUCTS. Naunyn, in the last century, assumed that lesions of the smallest bile ducts ("cholangie") were the basis for parenchymal jaundice in general, and much literature followed this concept. Eppinger spoke of a periacinous hepatitis with infiltration in this area [943]. Obstruction of the smallest bile ducts by mucopurulent plugs or fibrous scars in cholangitis is rare [1801]. Scarring or expansion of regenerating nodules in cirrhosis may compress the small ducts [1724, 3182], but no correlation can be demonstrated between the histologic appearance of liver biopsy specimens and functional evidence of cholestasis [2651]. Large or numerous regenerative nodules are often present without any evidence of cholestasis. Aplasia of the bile ducts is a rare and unquestionable example of mechanical intrahepatic obstruction [25] (see Mechanical Form, under Intrahepatic Cholestasis, later in this chapter).

OBSTRUCTION OF LARGE BILE DUCTS. Obstruction of one main hepatic duct or a smaller branch by gallstones or tumor metastases does not lead to jaundice, because the other lobe excretes the extra load of bile. Tumor nodules or stones within the liver, therefore, would have to occlude more than half of the bile ducts at the same time to produce jaundice. This is unlikely, and jaundice is often absent with almost complete replacement of the liver by tumor metastases. Jaundice in the presence of tumor metastases, therefore, is caused not by obstruction but rather by hepatocellular damage which is a reaction to tissue-breakdown products.

Functional Interference with Bile Flow. The inability to explain cholestasis without extrahepatic obstruction on a morphologic basis in many patients suggests that functional alterations are its cause. Three possibilities are discussed in detail.

HEPATOCELLULAR INSUFFICIENCY. Inability of the hepatic cells to secrete bile pigment into the bile canaliculi should result in a pigment-free white bile, which is described following reticulo-endothelial blockage [943]. "Paralysis" of bile formation or bilirubin removal [149, 1134] and reduction of the amount of parenchyma capable of secreting bilirubin [125] have been considered to be mechanisms of pigment accumulation. The presence of bile pigment in the cells and plugs in the bile canaliculi speaks against all these assumptions and indicates that bile pigment has passed through or between the hepatic cells into the bile canaliculi in nearly all instances of jaundice.

FUNCTIONAL INTERFERENCE WITH BILE CANALICULUS DRAINAGE. This may be caused by one of three factors:

1. Lack of secretory pressure. If the hepatic cells stop secreting, the forward movement of bile in the bile canaliculi ceases, and bile stagnates.
2. Excessive water resorption. This slows the rate of bile flow and increases the viscosity of the bile, further decreasing its rate of flow.
3. Lack of peristalsis. If contractibility of the bile canaliculi contributes to the forward movement of bile, its absence would explain impairment of bile flow. Emotional disturbances have been accused of producing or aggravating jaundice on this basis, but little support exists for acceptance of such a phenomenon.

ABNORMAL RESORPTION OF BILE PIGMENT. Morphologically intact hepatic cells or ductules can permit the regurgitation of bile or at least of bile pigments. In instances of intrahepatic cholestasis, bile is demonstrable through the entire length of the bile canaliculi up to the ductules, and the hepatic cells function normally. The site of regurgitation of biliary constituents has therefore been placed at the level of ductules [3510]. Biliary substances were assumed to leak back into the lymph or blood stream through morphologically intact epithelium. This concept led to the term "cholangiolitis," despite the fact that the inflammatory nature and the exact site of the process remain to be proved (see Cholangiolitis, Chap. 25).

Evaluation of Intrahepatic Cholestasis. Only in exceptional instances can mechanical factors explain the phenomenon of decreased or absent bile flow in the absence of extrahepatic obstruction. Functional factors, therefore, are more likely, at least initially, and of these, abnormal water resorption because of altered ductular permeability appears to be the most important (see Pathogenesis, under Intrahepatic Cholestasis, further on in this chapter). This militates against the use of the term, "intrahepatic biliary obstruction"; and the phenomenon is best designated "intrahepatic cholestasis."

PROPOSED CLASSIFICATION OF JAUNDICE

The criticisms presented can be leveled against almost every existing theory or classification of jaundice. This makes permissible the presentation of a more noncommittal but practical classification, which emphasizes a division of jaundice into

forms with and without impairment of bile flow (Fig. 73).

Jaundice without Impairment of Bile Flow

When the bilirubin in the blood stream exceeds the amount the liver is able to excrete, jaundice develops. Excessive formation of indirect-reacting bilirubin or a reduced threshold for hepatic removal accounts for the disparity.

Hemolytic (Overproduction) Jaundice. If bilirubin formation is excessive, the liver works at full

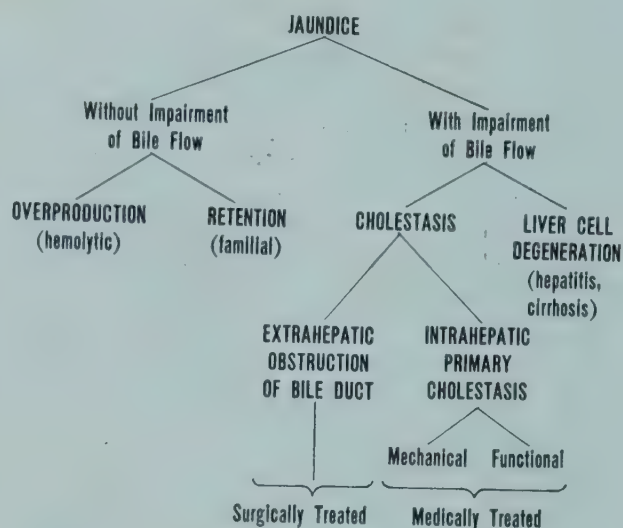


FIG. 73 Types of jaundice. Overproduction, retention, cholestasis, and liver cell degeneration are entities that can be differentiated by laboratory tests. (Popper, H., and Schaffner, F.: J.A.M.A. 150:1367, 1952.)

capacity, and although bilirubin in the bile is increased, it also accumulates in the blood. Functional impairment of the hepatic cells may be an added factor, because anemia is usually present. Since the excessively produced bilirubin in the blood has not been through the liver, it is indirect-reacting bilirubin, and no bilirubin appears in the urine (Fig. 74). The bile and the feces are dark, and the feces are rich in bilirubin. Greater than normal amounts of urobilinogen are formed and reabsorbed from the intestine. Although the hepatic cells reexcrete the greater portion of this into the bile, the urobilinogen concentration in the blood exceeds the overtaxed excretory ability of the liver, and urobilinogenuria results. Urinary urobilinogen is not necessarily elevated, however, despite much fecal urobilinogen; increased urinary urobilinogen may be a sign of a secondary hepatic-cell damage.

Many factors cause hemolysis. These include

(1) chemical or biological hemolysins, e.g., poisons, bacterial toxins, venoms; (2) immune hemolysins, e.g., in acute hemolytic anemia with or without cold agglutinins [999]; (3) immune agglutinins, e.g., in transfusion reactions and other blood group incompatibilities, such as erythroblastosis fetalis; (4) acquired hypersplenism, e.g., in splenomegaly from any cause; (5) congenital spherocytic anemia and the less common non-spherocytic form [2364]; (6) abnormal hemo-

about 7 mg per 100 ml. Subsequently it drops rapidly. Physiologic jaundice lasts from 3 to 7 days, seldom longer, and is not accompanied by any symptoms such as anemia or splenomegaly [738]. Jaundice appearing earlier is caused by hemolysis, while that appearing later or persisting beyond this time is the result of a variety of conditions (see Jaundice in the Neonatal Period, later in this chapter).

In premature infants with immature livers, physiologic jaundice appears earlier and persists longer, and the peak is higher and occurs later [1564]. Exceptionally, the clinical signs of kernicterus develop.

Nonhemolytic Retention Jaundice. A series of names has been given to an entity which possibly is a constitutional defect of the liver in the excretion of bilirubin. In effect, the threshold for the excretion of bilirubin into the bile is higher, although an anomaly of pyrrole metabolism with overproduction of bile pigment from nonhemoglobin precursors has been suggested [3638]. The pathogenesis of this phenomenon is still not clearly understood. It was described many years ago as "cholemie simple familiale." Subsequently, the differentiation from hemolytic jaundice seems to have been lost [943]. The name "chronic intermittent juvenile jaundice" was proposed, emphasizing its occurrence in young persons [2272], while its familial nature is stressed by the name "familial nonhemolytic jaundice" [715]. As the result of many reports [169, 497, 715, 2272, 2846], the disease entity appears well established, but borderline cases with almost physiologic jaundice seem to be rather common. In the liver of the newborn and especially of the premature infant, the threshold of bilirubin excretion is elevated and is responsible in part for neonatal jaundice. Exceptionally this may be a familial disease, with kernicterus, bile thrombi, and slight periportal fibrosis, and with a poor prognosis [688].

As in hemolytic jaundice, the bilirubin is chiefly indirect reacting. In contrast to the condition in familial hemolytic jaundice, the spleen is of normal size, spherocytosis and reticulocytosis are absent, red cell fragility is normal, urinary urobilinogen excretion is normal, fecal urobilinogen is lower than normal, and bilirubin tolerance is abnormal, suggesting faulty excretion of normal bilirubin by the liver. The results of hepatic-function tests are also normal, and histologically no abnormalities are noted [704, 1576, 1857].

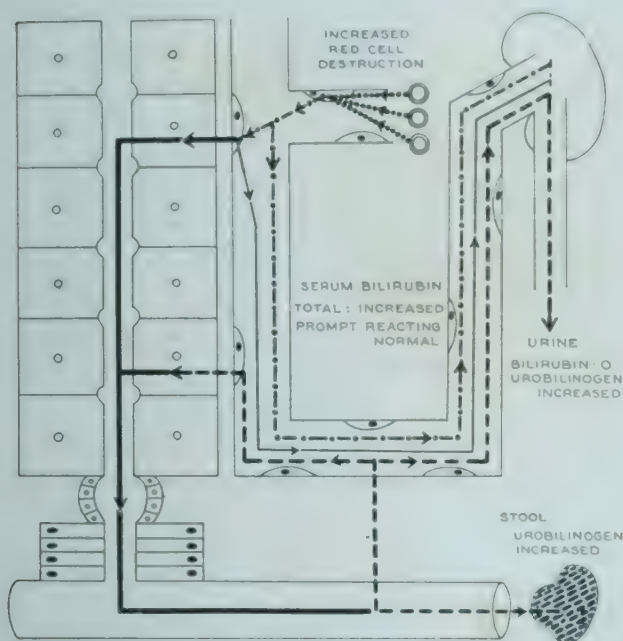


FIG. 74 Bile pigment metabolism in hemolytic jaundice. (Redrawn from Popper, H., and Schaffner, F.: *Advances Int. Med.* 4:357, 1950.)

globins, e.g., in sickle-cell anemia; (7) fetal hemoglobin in physiologic jaundice of the newborn; (8) unknown factors in such conditions as jaundice with hemoglobinuria [3173] and the symptomatic hemolytic anemias [3084].

PHYSIOLOGIC JAUNDICE, OR ICTERUS NEONATORUM. An extensive amount of literature exists concerning the mild jaundice in the neonatal period [3528]. It results mainly from two factors. One is the disposal of fetal hemoglobin [1926], and the second is the inability of the hepatic cells to excrete bilirubin rapidly, since the functional capacity of the liver is low at this age [3528]. The hemolytic effect of ingested fat in milk may also play a role [2047]. This jaundice develops on the second or third day of life in more than half of mature infants. The average total bilirubin of normal full-term infants is 2.0 mg per 100 ml at birth. It rises within 48 hours to an average of

The hyperbilirubinemia may be intermittent, and, in contrast to what happens in most other forms of jaundice, steroid hormones do not lower the level of the serum bilirubin [872].

Constitutional hepatic dysfunction [2846], which also seems to be hereditary in rats [2193], may be acquired in man, particularly following viral hepatitis [1576]. In the convalescent period after viral hepatitis, the level of indirect-reacting bilirubin may remain elevated for a considerable period of time without other clinical or histologic evidence of hepatic damage (see Persistent Hyperbilirubinemia, under Types of Protracted Hepatitis, Chap. 44) [1127, 1677]. This posthepatic disturbance of the hepatic bilirubin clearance is associated with as few clinical manifestations as are present in familial nonhemolytic jaundice. Therefore all cases of familial nonhemolytic jaundice may be sequelae of a preceding hepatitis [1576]. A type of mild jaundice has recently been reported that is characterized by impaired dye excretion and the appearance of an iron-free pigment in the hepatic cells, which otherwise appear normal and function normally (Dubin-Johnson syndrome). Bromsulphalein retention and non-visualization of the gallbladder on cholecystography are the only demonstrable functional abnormalities [848].

JAUNDICE WITH IMPAIRMENT OF BILE FLOW

Jaundice may result from impairment of the flow of bile either at its source (caused by hepatic-cell degeneration in hepatitis or cirrhosis) or during its course within the liver or in the extrahepatic bile ducts (caused by cholestasis). In all types of jaundice with impairment of bile flow, the levels of both the prompt-reacting and indirect-reacting bilirubin are elevated. As bilirubinemia develops, the predominant rise of the serum bilirubin initially occurs in the prompt-reacting fraction. When the total bilirubin level goes above 3.0 mg per 100 ml, the increases in the prompt-reacting and indirect-reacting fractions are parallel, regardless of the disease causing the impairment of the bile flow (Fig. 71).

If van den Bergh's concept of the transformation of indirect-reacting to prompt-reacting bilirubin by the liver is accepted, and no reason to discard this has been offered, the elevation of the total bilirubin level must be caused in part by

inability of the hepatic parenchyma to accept indirect-reacting bilirubin, since regurgitation could account only for elevation of the prompt-reacting fraction. Some prompt-reacting bilirubin is normally in the blood stream. The transformation possibly occurs somewhere between the Kupffer and hepatic cells. The elevation of both indirect-reacting and prompt-reacting bilirubin in jaundice suggests the presence of a Kupffer cell-hepatic-cell block, with escape of prompt-reacting bilirubin from the Kupffer cells back into the blood stream, either directly or via the tissue spaces and lymphatic vessels [2906]. This block is subsequently followed by nonacceptance of indirect bilirubin, probably because the transforming mechanism is saturated with bilirubin. This saturation is recognized histologically by bile inhibition of the Kupffer cells. The Kupffer cell-hepatic-cell block may be caused by either hepatocellular degeneration or bile stasis. It may be the result of depression of a specific enzyme necessary for the transfer of bilirubin from the blood stream to the bile canaliculi [1375].

The possibility of a Kupffer cell-hepatic-cell block does not exclude the existence of regurgitation, through either hepatic cells or ductules. Regurgitation seems best to explain the increase in the blood of biliary substances other than bilirubin, such as alkaline phosphatase, cholesterol, bile acids, and phospholipids.

Hepatocellular Degeneration

Jaundice from hepatocellular degeneration is associated with morphologically demonstrable injury of hepatic cells. This occurs not only in primary hepatitis and cirrhosis, but also when hepatocellular degeneration complicates other conditions such as biliary obstruction, passive congestion, anoxia, etc. It is best explained by the presence of a Kupffer cell-hepatic-cell block. In some stages, regurgitation through broken hepatic-cell plates from the bile canaliculi into the tissue space has also been assumed, especially if no bile plugs are noted, as in early stages of viral hepatitis [3093]. In hepatocellular degeneration, the total serum bilirubin level and the prompt-reacting fraction are elevated, and bilirubin appears in the urine (Fig. 75). Less bilirubin than normal enters the gastrointestinal tract, where less urobilinogen than normal is formed, and the feces appear light but not acholic. Less urobilinogen than normal is reabsorbed, but the hepatic-cell damage prevents

its reexcretion through the liver, an interruption of the enterohepatic circulation, and more urobilinogen than normal appears in the urine.

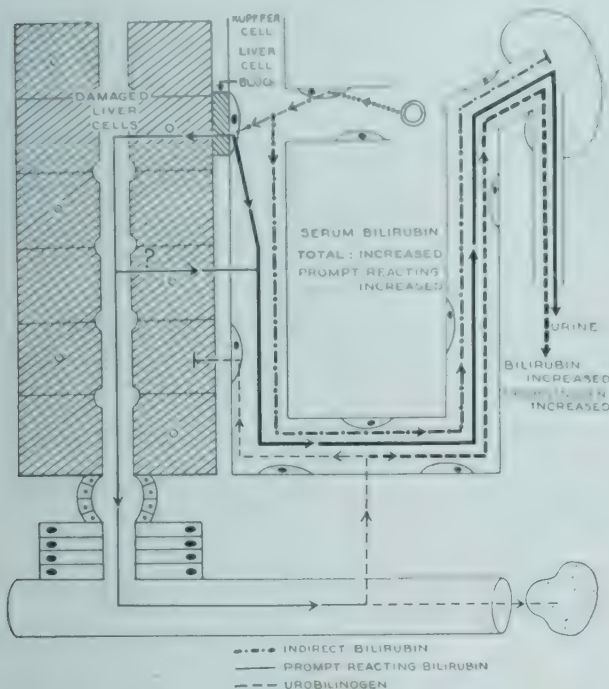


FIG. 75 Bile pigment metabolism in hepatocellular degeneration. (Redrawn from Popper, H., and Schaffner, F.: *Advances Int. Med.* 4:357, 1950.)

Cholestasis

Impairment of bile flow by mechanical factors or as the result of a functional disorder of the ductules for which clear-cut anatomical evidence can not be presented is called "cholestasis." Jaundice from cholestasis has either intrahepatic or extrahepatic causes. Extrahepatic causes are always mechanical, whereas intrahepatic causes may be either mechanical or functional. The jaundice may be caused by a Kupffer cell-hepatic-cell block caused by the stagnation of the flow of bile, with the increased pressure stopping the secretion of bile by the hepatic cells or suppressing a specific enzyme system [1375].

Vital microscopy in animals with mechanical biliary obstruction shows that bile canaliculi do not necessarily dilate when bile flow stops. This suggests metabolic reasons, rather than mechanical ones, for the development of jaundice in obstruction [1242]. In addition, regurgitation of bile from the biliary passages must be considered. The intact appearance of the hepatic-cell plates in all types of cholestasis [1566] speaks against regurgitation through hepatic cells. The site of

regurgitation has been customarily located in the ductules [1903, 3510].

Anatomically recognizable rupture in this location causes "bile lakes" (see Bile Stasis and Extravasation, Chap. 24). Regurgitation of biliary substances results in periductular inflammation and, in protracted cholestasis, in fibrotic strangulation, with the development of an intrahepatic mechanical component to the cholestasis. Regurgitation is the usual explanation advanced for the increase of biliary substances, other than bilirubin, in the blood stream. This increase may also result from metabolic alterations in the presence of cholestasis, with subsequently increased formation of these substances (see Specific Alterations of Hepatic Function, under Functional Consequences, in Chap. 24).

Extrahepatic Cholestasis. To produce jaundice, extrahepatic biliary obstruction has to involve the main axis of the bile flow, the main hepatic or common duct, anywhere from the junction of the hepatic ducts at the hepatic hilus to the papilla of Vater. An obstacle, usually a tumor, stone, stricture, atresia, parasite, or spasm, interferes with the free flow of bile into the duodenum and causes a dilatation of bile ducts above the obstruction. The extrahepatic bile ducts are dilated above the obstruction, in contrast to the narrow ducts in intrahepatic cholestasis. This basic difference is readily apparent on surgical exploration, with or without cholangiography. The obstruction may be complete, incomplete, or intermittent.

Bile Pigment Metabolism in Extrahepatic Cholestasis. In both complete and incomplete obstruction, normal formation of indirect-reacting bilirubin occurs. Both indirect-reacting and prompt-reacting bilirubin levels are elevated, however, and bilirubin appears in the urine owing to the presence of a Kupffer cell-hepatic-cell block, as well as to regurgitation. In complete obstruction, no bile reaches the intestine, no urobilinogen is formed, the stools are acholic, no urobilinogen is reabsorbed, and none is found in the urine (Fig. 76). The picture of persistent complete obstruction is characteristic for tumors, where a gradual reduction of the urobilinogen excretion typically occurs simultaneously with an increase of the serum bilirubin.

In incomplete obstruction, some bilirubin enters the intestine, some urobilinogen is formed and the feces are light but not acholic (Fig. 77). Less urobilinogen than normal is reabsorbed, but

since the enterohepatic circulation is interrupted because of hepatic-cell degeneration, more urobilinogen than normal is excreted in the urine. In patients with stones and occasionally in those with strictures the intermittent obstruction is reflected in spiking urinary urobilinogen and bilirubin concentrations [3185] (Fig. 78, top).

Causes of Extrahepatic Biliary Obstruction. **TUMORS.** Obstruction of the biliary tract by tumor may result from (1) primary intrinsic tumor of the

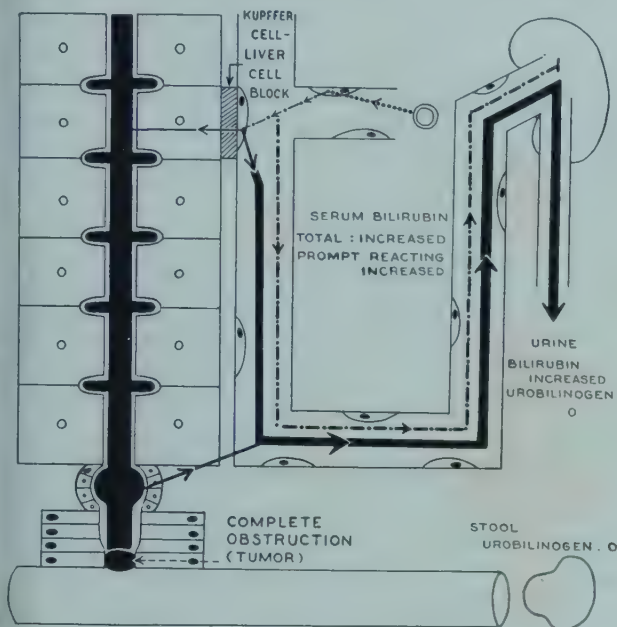


FIG. 76 Bile pigment metabolism in extrahepatic cholestasis (complete biliary obstruction). (Redrawn from Popper, H., and Schaffner, F.: *Advances Int. Med.* 4:357, 1950.)

biliary ducts; (2) primary tumors in the structures surrounding the ducts, compressing and often invading them; (3) metastatic tumors in or around the ducts.

Intrinsic Tumors. The primary intrinsic tumors may be benign or malignant. The benign tumors are rare and are most often adenomas, epithelial in origin. The malignant tumors are carcinomas of the biliary duct or papilla of Vater. They also include carcinoma arising at the bifurcation of the common hepatic duct, since the findings are those of an extrahepatic lesion, although it is actually intrahepatic. The obstruction rapidly becomes complete, and only rarely does it give way temporarily to allow uninhibited bile flow, as, for instance, when a carcinoma of the papilla of Vater becomes necrotic and part of the obstructing mass is sloughed. This is usually associated with an episode of melena.

Primary Tumors Surrounding Bile Ducts. Obstruction results from tumors in surrounding structures such as carcinoma of the head of the pancreas, the duodenum, or the gallbladder, if the tumor has invaded the wall of the bile duct so as to fix it [1685] (Fig. 79). A duct which is not fixed may move aside, despite enlargement of the tumor itself. Jaundice in lymphomas or other conditions associated with enlargement of the portal lymph nodes but without fixation of the common duct is usually not the result of mechanical obstruction but of processes involving the liver parenchyma. Obstruction is produced only in exceptional cases by discrete lymph node tumors, such as occur in leukemia, tuberculosis, or Hodgkin's disease [360].

Metastatic Tumors. Metastatic obstruction may be caused by tumor deposits within the wall of the ducts, within adjacent lymphatic vessels, or

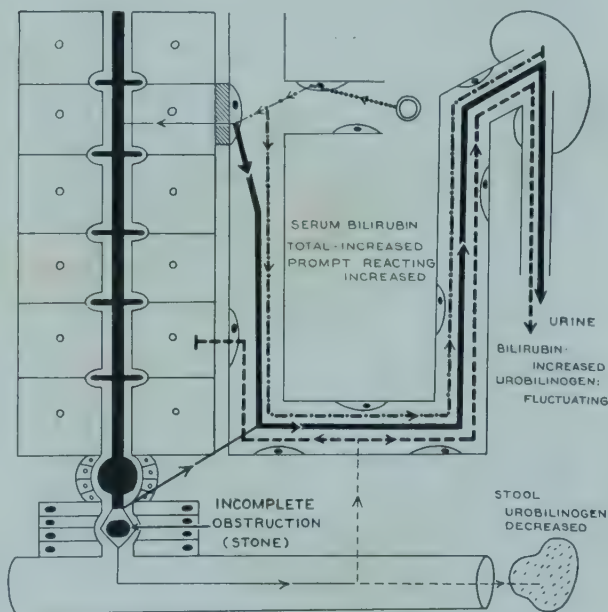


FIG. 77 Bile pigment metabolism in extrahepatic cholestasis (incomplete biliary obstruction). (Redrawn from Popper, H., and Schaffner, F.: *Advances Int. Med.* 4:357, 1950.)

within the areolar tissue of the hilus. In involvement of the periportal lymph nodes, the metastatic lesion has to fix the wall of the duct by invasion in order to compress it. Metastases to portal lymph nodes occur frequently but produce biliary obstruction infrequently, although not so rarely as some recent reports have indicated [1465] (Fig. 80). The primary sites of tumors producing biliary obstruction as a result of lymph node metastases are the tail of the pancreas, the

stomach, the colon, the ovaries, and the bronchi, in the order of their frequency, although any other tumor may potentially cause such obstruction.

STONES. Calcareous obstruction is usually complete initially, since the spasm and edema associated with early impaction of a gallstone in the common duct prevent any discharge of bile. Subsequently, with relaxation of the spasm, the obstruction becomes incomplete because of the elasticity and pliability of the duct wall and the dilatation of the duct. Further movement of the

stone may again lead to complete obstruction or at least to a ball-valvelike effect (Fig. 81, 82). The alternation of short periods of complete obstruction with periods of increased release of bile is characteristic for stones and is reflected in the fluctuations in the urinary urobilinogen and bilirubin concentrations (Fig. 78, top). The initial period of complete obstruction by stones may be prolonged if an inflammation around the stone replaces the spasm. Stones in the terminal portion of the cystic duct partially covered by mucosa

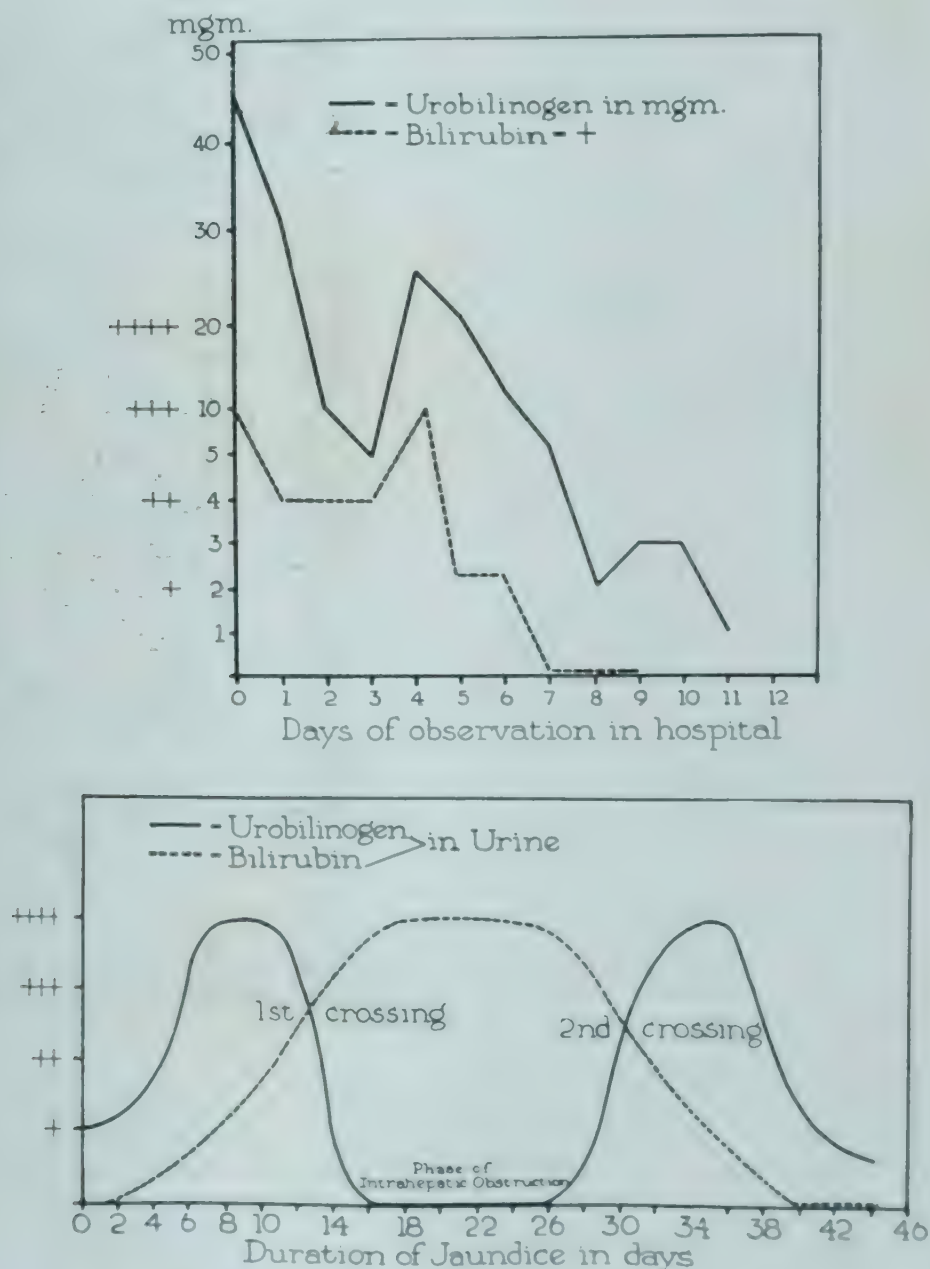


FIG. 78 Upper. Quantitative urobilinogen excretion in a case of incomplete obstructive jaundice due to common duct stone. Lower. Schematic curve of the results of qualitative analysis for bilirubin and urobilinogen in a typical case of parenchymatous jaundice with temporary cholestasis (Steigmann, F., Popper, H., and Meyer, K. A.: J.A.M.A. 122: 279, 1943.)

may protrude into the lumen of the hepatic duct and produce incomplete obstruction (Fig. 81).

STRICTURES. Primary strictures produced by inflammatory scarring are rare and usually cause incomplete or occasionally fluctuating obstruction. In spontaneous benign stenosis, dense fibrous thickening of the duct occurs without increasing its diameter. This is seen on the upper part of the common duct or in the hepatic duct [508, 687].

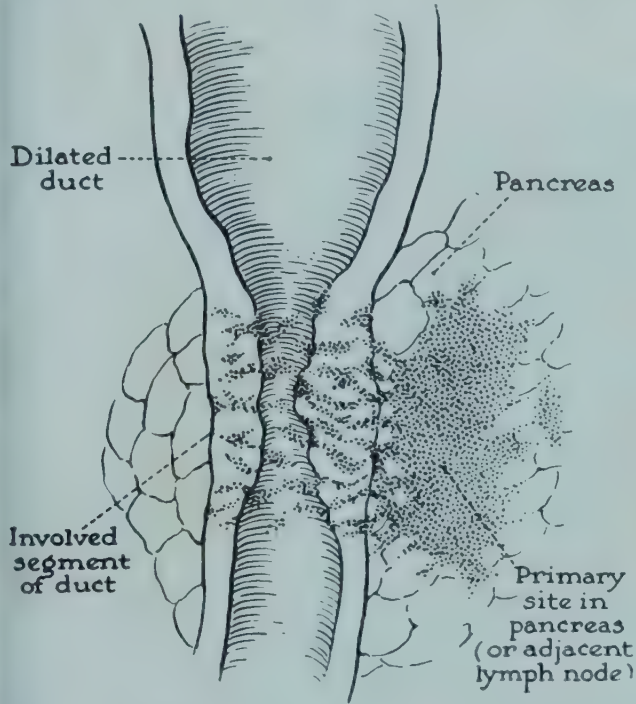


FIG. 79 Drawing of obstruction of common duct as a result of carcinomatous invasion of its wall. (Kaplan, N., and Angrist, A., by permission of *Surgery, Gynecology, and Obstetrics* 77:199, 1943.)

Strictures occasionally result from decubital ulcers owing to choledocholithiasis. The great majority of acquired strictures develop after cholecystectomy [1901, 2115] and are caused by crushing of the duct by clamps in hasty efforts to control bleeding. Recurrent jaundice, often with chills and fever, occurs in such patients, usually 3 to 4 months after surgery, when progressive scarring has led to deformity and constriction of the duct.

ACCIDENTAL SURGICAL INTERRUPTION OF THE COMMON OR HEPATIC DUCT. This results either from confusion of the cystic duct with the common or hepatic duct or from anomalies in the arrangement and number of the ducts [1901]. A portion of the duct is often excised. A spontaneous external biliary fistula immediately forms, which closes spontaneously after several months. At that time jaundice develops. It usually subsides, with

the feces becoming brown in color, following the formation of an internal biliary fistula between the dilated stump of the duct and the adjacent and adherent duodenum. These fistulas rarely remain patent and usually contract to a point where eventually no bile passes through them and permanent, complete extrahepatic biliary obstruction develops, requiring surgical repair.

STENOSIS FROM INFLAMMATION IN NEIGHBORING ORGANS. Inflammatory [633] or cystic lesions [1666] in the pancreas may result in stenosis or compression of the common bile ducts. In some in-

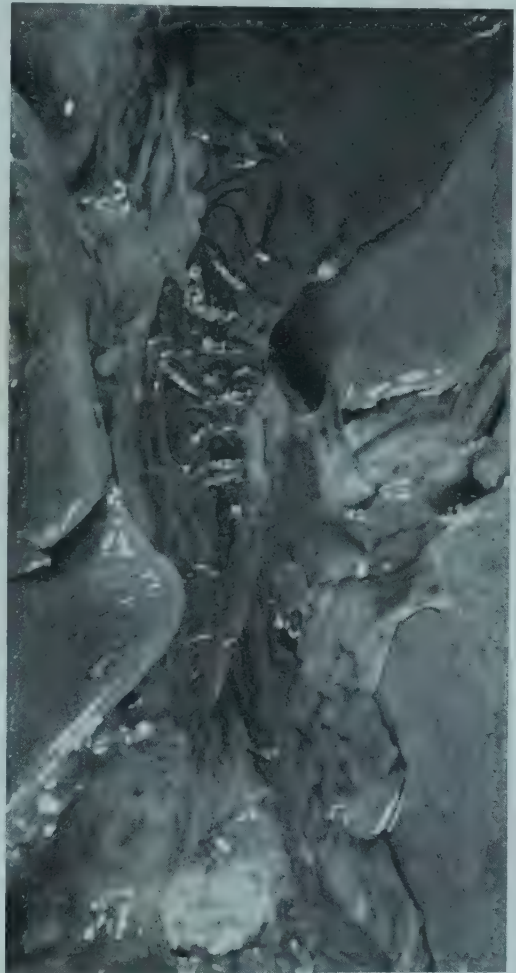


FIG. 80 Obstruction of terminal portion of common duct by peripancreatic carcinomatous lymph nodes fixing the duct by invasion (in a patient with carcinoma of the colon). Dilatation of gallbladder and biliary ductal system.

stances pancreatic duct calculi cause compression of the common bile duct or obstruction at the ampulla of Vater. Transient postoperative pancreatitis after biliary tract surgery, especially following intubation of the ducts, also can cause

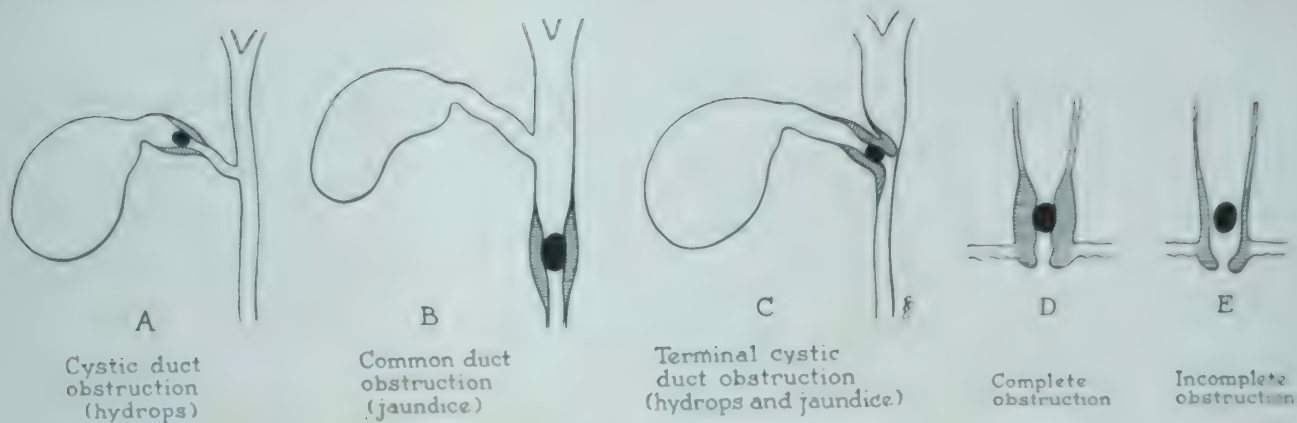


FIG. 81 Various types of biliary ductal obstruction by calculi.



FIG. 82 Cholangiograms showing various degrees of obstruction of the common bile duct caused by stones. *Upper left.* Complete obstruction by stone at end of common duct near bottom of picture. *Upper right.* Incomplete obstruction by stone at end of common duct showing escape of radiopaque material into duodenum. *Lower.* Serial pictures showing ball-valve-like action of a group of stones, with complete obstruction when the stones are impacted in the lower end of the duct (lower right picture). (Courtesy of Dr. Lillian Donaldson.)

jaundice. This condition, however, is usually the result of associated hepatic-cell degeneration. Inflammatory reactions around duodenal ulcers [1974] or diverticula in the second portion of the duodenum produce obstructive jaundice in rare instances.

ATRESIA. Congenital atresia of the bile duct usually produces complete obstruction (see Atresia, under Biliary System, Chap. 20).

PARASITES. Parasites in the bile ducts, particularly *Clonorchis sinensis*, *Fasciola hepatica*, or *Ascaris* [3054], result in biliary obstruction which is rarely complete.

SPASM. French [2544] and German authors claim that spasm of the sphincter of Oddi, possibly caused by duodenitis, produces jaundice. The evidence for this claim is not convincing.

OTHER CAUSES OF OBSTRUCTION. Foreign bodies, such as bullets or surgical instruments, have been found obstructing the common bile duct [1440]. Whether mucous plugs cause obstruction in infants is questionable (see "Inspissated Bile Syndrome," under Extrahepatic Bile Ducts, Chap. 20). Peritoneal adhesions rarely result in obstruction. In vitamin A-deficient rats detached masses of biliary epithelial cells obstruct the common duct [1368].

Intrahepatic Cholestasis. Intrahepatic cholestasis must be diffuse in order to produce jaundice. Diffuse cholestasis in the larger bile ducts, similar to that in extrahepatic obstruction, results from intrahepatic causes only in carcinomas involving the bifurcation of the hepatic duct, or possibly in purulent cholangitis. Whether jaundice in cholangitis is caused by purulent obstruction per se, by accompanying stones, or by hepatic-cell degeneration is questionable. Infiltration by primary or secondary carcinomas or by lymphomas produces local cholestasis, recognizable in biopsy specimens. Multiple and extensive tumorous infiltrations are frequently associated with jaundice, although intrahepatic cholestasis does not suffice to produce jaundice. It may, however, be a component responsible for the functional cholestatic features, such as elevation of the serum-alcaline phosphatase activity. This cholestasis is usually superimposed on the effects of loss of hepatic parenchyma and the toxic effects of tissue-breakdown products, accounting for the signs of hepatocellular degeneration such as the abnormal serum-protein reactions.

In the evaluation of intrahepatic cholestasis involving the lobular parenchyma and ductules,

mechanical and functional factors must be separated.

MECHANICAL FORM. A typical example of primary, mechanical intrahepatic cholestasis is congenital aplasia of the ducts in the portal tracts [25]. A mechanical obstruction in hepatitis or cirrhosis and even in prolonged extrahepatic cholestasis occurs as a result of fibrotic strangulation of the ductules secondary to inflammation and functional intrahepatic cholestasis.

FUNCTIONAL INTRAHEPATIC CHOLESTASIS ("CHOLANGIOLITIS"). Complete or incomplete interruption of the bile flow can be demonstrated in some patients in whom anatomic investigation at operation or necropsy fails to implicate a site or mechanism, the extrahepatic bile ducts being patent and normal in caliber. Nevertheless, the histologic findings in the liver are the same as in extrahepatic mechanical biliary obstruction. Inflammatory exudate is usually found in the portal tracts or around the intralobular ductules, but the incidence or extent of the exudate in no way parallels the degree of jaundice, for instance, in viral hepatitis [2189] or in cirrhosis [2651].

The argument concerning this puzzling type of jaundice has been reviewed (see earlier in this chapter). Biochemical observations and the anatomic finding of bile plugs up to but not beyond the intralobular and periportal ductules link this type of jaundice with a functional alteration of the ductules [943, 1903, 2797, 3510]. The clinical and laboratory picture is best reconciled with the assumption of increased permeability of the ductules, permitting bile to flow back through ductular epithelium into the surrounding tissue, with subsequent regurgitation into the blood. More water than biliary substances may flow back, resulting in an inspissation of biliary solids, which in turn produces bile stasis, recognizable by bile casts. In most instances some bile continues to pass through the ductules into the bile ducts, and the interference with the bile flow is incomplete. In a few instances, however, the combination of stasis and back flow of bile is severe enough to interrupt the bile flow completely and to produce a condition which clinically and biochemically resembles complete biliary obstruction and for which the name "intrahepatic cholestasis" is preferred (see Evaluation of Intrahepatic Cholestasis, earlier in this chapter). The frequency of inflammatory exudate around the ductules in such instances has also given rise to the term "cholangiolitis" [2797, 3510] for this lesion, although

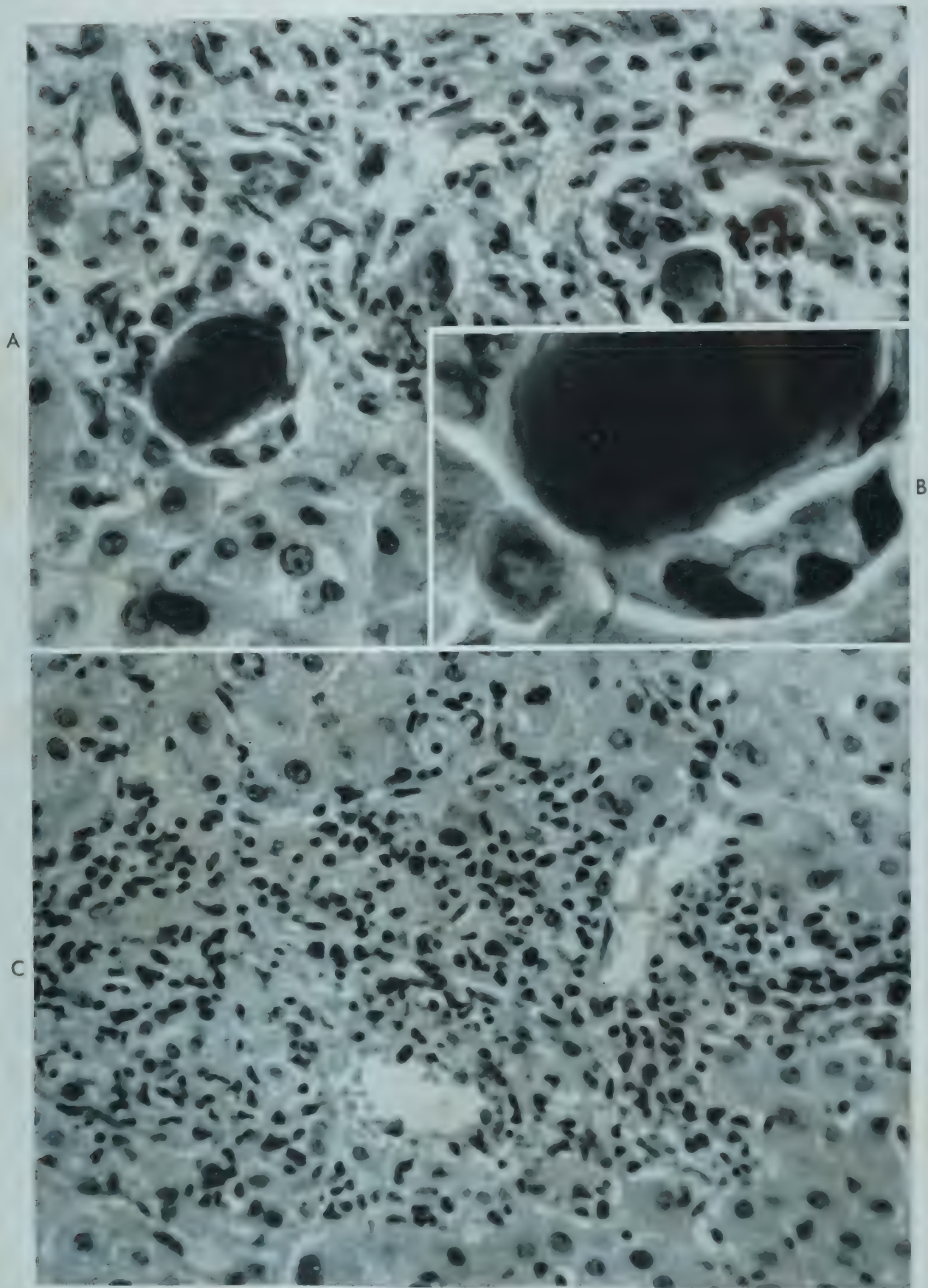


FIG. 53. *A.* Microcalculi in dilated ductules surrounded by inflammatory exudate in fatal viral hepatitis. The bile plugs are only in part surrounded by ductular epithelium, which is thin in some places and proliferated in others. H&E ($\times 330$). *B.* Enlargement of portion of bile plug in *A* to show the plug adjacent to the basement membrane and not covered by epithelium. H&E ($\times 630$). *C.* Allergic cholangiolitis associated with treatment using para-aminosalicylic acid. Inflammatory exudate containing some eosinophils in the portal tract and periportal zone surrounds a few proliferated ductules. H&E ($\times 230$).

the inflammatory component is probably secondary.

Pathogenesis. As the cause of functional intrahepatic cholestasis, present evidence points to altered ductular permeability, comparable to the alteration of the renal tubules seen in the nephrotic stage of glomerulonephritis, permitting abnormal reabsorption of urinary substances, often without morphologic changes [996]. The perilobular and intralobular ductules are the site of this increased permeability, especially in autopsy specimens. Inspissation of bile, forming thick plugs, or microcalculi, can be seen (Fig. 83A, B), associated with thinning or disappearance of the lining epithelium (see Early Cholestasis, under Intrahepatic Cholestasis, Chap. 24). In larger intraductular bile plugs, globular areas of increased density can sometimes be noted reflecting a greater degree of inspissation. In subacute or chronic stages the number of intralobular and perilobular ductules appears increased, and they are surrounded by inflammatory exudate. The exudate, which irritates the periductular tissue, is probably secondary, and results from regurgitated biliary substances. Protracted exudation leads to periductular fibrosis. The increase in the number of ductules has usually been considered proliferation of preexisting ductules. However, the embryologic observations [1545] that ductules develop from hepatic cells (see Embryology, Chap. 20) support the hypothesis that the increased number of visible ductules results from transformation from hepatic cells. This transformation may parallel an increase in the permeability of preexisting or newly transformed ductules. Apparently the size of the ductular cells and the width of the ductular lumens show individual variations, which, associated with increased permeability, may be the initial abnormality, with regurgitation anatomically not apparent. The injury may be either a specific effect upon the ductules, similar to that on the hepatic cells, as in viral hepatitis, or it may result from excretion of irritating material in the bile. The presence of eosinophils in the periductular exudate in drug-induced hypersensitivity lends credence to the latter assumption (Fig. 83C).

As a result of cholestasis in the liver, bile also becomes inspissated in larger bile ducts, leading to precipitation of biliary components, with the formation of biliary sludge or sand in protracted cholestasis from any cause. This usually has no mechanical effects; the bile ducts are not dilated,

and the inspissated bile does not contribute to the jaundice. Exceptionally, choledocholithiasis develops, with extrahepatic biliary obstruction, as a late complication of chronic intrahepatic cholestasis. This may result in the late appearance of biopsy findings of extrahepatic cholestasis, such as extravasation of bile.

In conclusion, the pathogenesis of intrahepatic cholestasis as an independent disease or as a component of other diseases is not established, but the evidence points to a primary increase in permeability of preexisting ductules and those newly transformed from hepatic cells, resulting in periductular inflammation and even fibrosis.

Disturbances of Bile Pigment Metabolism in Intrahepatic Cholestasis. The formation of bilirubin from hemoglobin is normal. Prompt-reacting bilirubin which reaches the ductules regurgitates into the tissue spaces and the blood stream. In addition, the inspissation of bile results in bile stasis and the development of a Kupffer cell-hepatic-cell block. This in turn leads to increased amounts of prompt-reacting bilirubin returned to the blood stream and nonacceptance of indirect-reacting bilirubin. The levels of both bilirubin fractions rise, and bilirubin appears in the urine. In exceptional instances, inspissation and regurgitation from the ductules are severe enough to prevent bile from reaching the intestine. In these instances, the feces are acholic and no urobilinogen is found in the urine. In most cases, some bilirubin enters the intestine, some urobilinogen appears in the urine, and the feces are not completely acholic (Fig. 84).

Causes of Intrahepatic Cholestasis. Intrahepatic cholestasis seldom occurs in a pure form and rarely is the sole reason for jaundice. It is a frequent component in diseases with primary hepatocellular degeneration. In such instances, especially with severe jaundice, the contributions of hepatocellular degeneration and of cholestasis are difficult to evaluate. Functionally, the cholestatic component is indicated by increase of serum-alkaline phosphatase activity, elevation of total cholesterol and phospholipid levels, and reduction or absence of urinary urobilinogen.

VIRAL HEPATITIS. In many instances of viral hepatitis, morphologic and functional evidence of cholestasis is found. Cholestasis is a frequently found phase in severe hepatitis and is best reflected by temporary reduction or even disappearance of urinary urobilinogen while urinary bilirubin is high (Fig. 78, lower). Instances occur,

however, in which cholestasis dominates the picture, either from the beginning or in later stages. Many cases of the so-called cholangiolitic hepatitis belong in this group [26, 3510].

POISONS. Experimental cholangiolitic changes, with or without functional evidence of bile stasis, have been produced by various substances, such as toluylenediamine [325], manganous chloride, alpha-naphthylthiourea (ANTU) [2001], and ethionine [2635]. These findings have prompted the use of the designation "cholangiotoxic lesion" [2797].

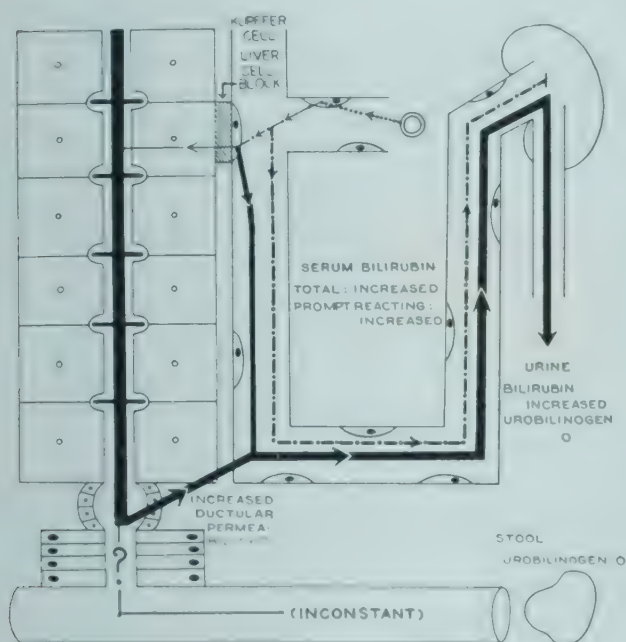


FIG. 84 Bile pigment metabolism in intrahepatic cholestasis (complete obstruction). (Redrawn from Popper, H., and Schaffner, F.: *Advances Int. Med.* 4:357, 1950.)

HYPERSENSITIVITY DRUG REACTIONS. In some patients various drugs, such as methyltestosterone [397, 1767, 3559], para-aminosalicylic acid (PAS), arsenicals [1092, 1377, 2046, 3236], and chlorpromazine produce intrahepatic cholestasis. In some instances, severe morphologic changes and increase in alkaline phosphatase activity occur without much jaundice, while in others, jaundice is very severe without any conspicuous morphologic changes of the ductules, as in methyltestosterone jaundice. Only some patients receiving these drugs show the morphologic changes, and readministration after subsidence of symptoms is usually not followed by recurrence of jaundice. These observations, supported by the frequent finding of eosinophils in the liver and sometimes in the circulating blood, suggest a hypersensitivity

reaction around the ductules, rather than metabolic hepatocellular alteration without visible change [1767].

NEONATAL JAUNDICE. Jaundice appearing in the first or second week of life and persisting or even increasing in intensity is sometimes due to intrahepatic cholestasis. In such instances the extrahepatic biliary tree is normal, with small amount of inspissated bile seen in the extrahepatic bile ducts (see "Inspissated Bile Syndrome," under Biliary System, Chap. 20).

BACTERIA. Bacterial cholangiolitis was often assumed in previous years (see Bacterial Cholangiolitis, under Cholangiolitis, Chap. 25).

CIRRHOSIS. In various types of cirrhosis, including those belonging to the nutritional fatty-liver-cirrhosis syndrome, some intrahepatic cholestasis may be found. It is observed with some frequency both in fatty liver with hepatic failure and in florid cirrhosis. In some relatively rare types of cirrhosis intrahepatic cholestasis is the main feature. Their etiology is not established, although some types seem to result from a preceding viral hepatitis, while others are possibly late stages of a hypersensitivity reaction. This lesion, which also occurs in a subacute form [2767], has been considered a primary connective tissue proliferation and not a reparative response [2153]. It is frequently associated with hypercholesteremia and subsequent xanthoma formation, and has been called "xanthomatous biliary cirrhosis," "pericholangiolitic biliary cirrhosis" [2156], "primary biliary cirrhosis" [27], or "cholangiolitic cirrhosis" [1696, 2797] (see "Xanthomatous Form" of Cholangiolitic Cirrhosis, under Cholangiolitis and Pericholangiolitis, Chap. 46). The cirrhosis of Hanot also belongs to this group.

Discussion of the Proposed Classification

A classification of jaundice finds its justification in the practical clinical application. The pathogenesis of the pure forms is summarized for review in Table 9. Jaundice without impairment of bile flow is identified by laboratory tests. It is characterized by a normal prompt-reacting serum bilirubin level and absent bilirubinuria. Overproduction jaundice is recognized by the laboratory findings of hemolysis and is a hematologic problem. The management, including the decision as to whether splenectomy is indicated, is based on hematologic considerations.

In contrast, the management of the forms of jaundice associated with impairment of bile flow

is decided mainly on the basis of altered hepatic function and structure.

Table 9 Pathogenesis of Jaundice

Pure Forms of Jaundice	Pathogenesis
Retention.....	Increased threshold for bilirubin excretion
Overproduction.....	Increased red cell destruction (hemolysis)
Hepatocellular degeneration....	Bilirubin not accepted by hepatic cell because of liver damage and either returned to blood stream or not accepted by Kupffer cell (Kupffer cell-hepatic cell block)
Extrahepatic cholestasis.....	Kupffer cell-hepatic cell block because of increased biliary pressure arresting bilirubin excretion; also regurgitation from ductules with subsequent periductular inflammation
Intrahepatic cholestasis.....	Increased permeability of ductules with inspissation of bile and increase in biliary pressure causing Kupffer cell-hepatic cell block; also regurgitation from ductules with periductular inflammation leading to strangulation, further increasing biliary pressure

In most examples of jaundice with impairment of bile flow, different factors cooperate to various degrees to account for the great variety of the picture. In jaundice from intrahepatic lesions, four of these factors may be present, two of which are more important. The more common factors are hepatic-cell degeneration and intrahepatic cholestasis on a functional basis (cholangiolitis). In rare instances, only one of the two may be present, whereas in most clinical examples, both exist to various degrees (Fig. 85). The two other com-

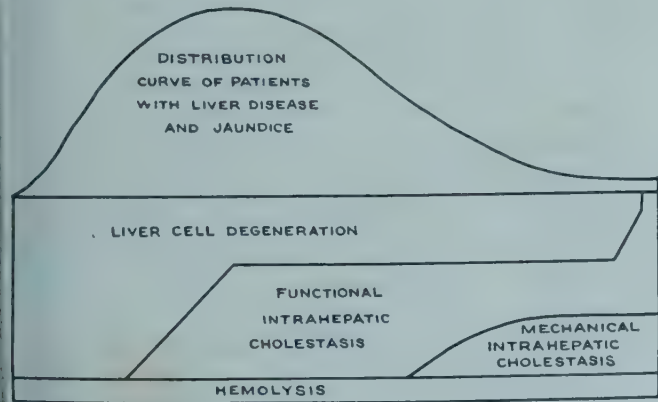


FIG. 85 Contribution of various factors to the pathogenesis of jaundice as a result of liver disease, with a theoretical curve of the relative incidence.

ponents adding to the jaundice are overproduction of bilirubin, or hemolysis, and intrahepatic cholestasis on a mechanical basis as a result of strangulation of the bile duct by scar tissue or inflammatory exudate. In jaundice from an obstruction of the extrahepatic biliary tract, hepatic-cell degeneration and sometimes hemolysis are present, in addition to extrahepatic cholestasis (Fig. 86). In addition, periductular exudate suggests that me-

chanical intrahepatic cholestasis is a complication, especially in chronic biliary hepatitis.

“Surgical” and “Medical” Jaundice. The therapeutic indications, namely, the division into surgical and medical jaundice, in practice probably the most important differential diagnosis, do not parallel the laboratory classification presented (Fig. 73). This fact is the source of great diagnostic difficulty in the application of the laboratory tests. In general, medical jaundice, mainly from hepatitis and cirrhosis, includes not only hepato-

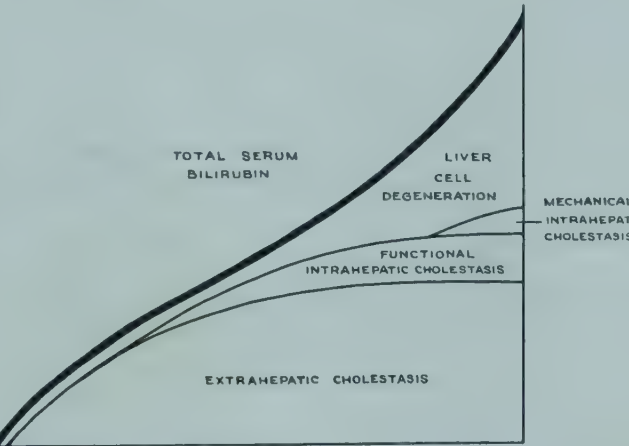


FIG. 86 Factors that contribute to the elevation of the serum bilirubin in extrahepatic cholestasis. Complete extrahepatic obstruction is responsible for elevation of the bilirubin level to about 15 mg per 100 ml serum.

cellular degeneration but also intrahepatic cholestasis. The latter presents great difficulty in the differential diagnosis of surgical and medical jaundice by laboratory tests, followed in importance by the hepatocellular damage secondary to extrahepatic biliary obstruction or to superimposed bacterial infection. In the discussion of the clinical differential diagnosis, these points find special consideration (see Outline for Laboratory Differential Diagnosis of “Medical” and “Surgical” Jaundice, Chap. 65).

Jaundice in the Neonatal Period. Jaundice in the neonatal period presents a special diagnostic problem deserving review. It is the most frequently encountered form of jaundice. Since it is to some degree caused by immaturity of the liver and is related to disposal of fetal hemoglobin, it is called “physiologic jaundice,” or “icterus neonatorum” (see Physiologic Jaundice, or Icterus Neonatorum, earlier in this chapter). Bilirubin levels over 10 mg per 100 ml serum which do not drop within 4 days are abnormal [1564]. As in adults, jaundice in the neonatal period may result from

(1) overproduction, (2) retention, (3) hepatocellular degeneration, (4) cholestasis. It may also be caused by combinations of these processes. The distribution differs from the adult, with predominance of overproduction (hemolytic) jaundice. The excessive amount of indirect-reacting bilirubin in the serum in jaundice without impairment of bile flow causes kernicterus (see Kernicterus, under Influence of the Liver on the Brain, Chap. 63), which is absent in jaundice with impairment of bile flow. Since the crippling neurologic manifestations of kernicterus are of great importance in neonatal jaundice, the differential diagnostic classification of this disorder is presented.

I. Overproduction (hemolytic) jaundice

- A. Physiologic jaundice (icterus neonatorum)—most common (see this heading, earlier in this chapter)
- B. Hemolytic disease from sensitization of mother (erythroblastosis fetalis)—frequent (see Hemolytic Disease of the Newborn, Chap. 49)
- C. Congenital familial hemolytic anemia—rare (see Congenital and Acquired Hemolytic Anemia, Chap. 49)
- D. Sepsis?
- E. Blood dyscrasias—rare

II. Retention

- A. Jaundice of prematurity—rare (see Physiologic Jaundice, or Icterus Neonatorum, earlier in this chapter)
- B. Congenital familial nonhemolytic jaundice—rare (see Nonhemolytic Retention Jaundice, earlier in this chapter)

III. Hepatocellular degeneration

- A. Viral, or giant-cell, hepatitis, usually associated with intrahepatic cholestasis—frequent (see Giant-cell Hepatitis, Chap. 46)
- B. Congenital luetic hepatitis—now rare (see Hepatic Syphilis, Chap. 54)
- C. Galactosemia—rare (see Galactosemia, Chap. 53)
- D. Cytomegalic inclusion disease—rare (see Cytomegalic Inclusion Hepatitis, under Other Types of Hepatitis Produced by Viruses, Chap. 45)
- E. Herpes simplex hepatitis—rare (see Herpes Simplex Hepatitis, under Other Types of Hepatitis Produced by Viruses, Chap. 45)
- F. Sepsis, including umbilical vein sepsis—now rare (see Bacterial Hepatitis, under Nonspecific Reactive Hepatitis, Chap. 41)

IV. Cholestasis

- A. Congenital extrahepatic biliary obstruction ("surgical jaundice" of the newborn)—not rare (see Extrahepatic Bile Ducts, under Biliary System, Chap. 20)

- B. Congenital intrahepatic biliary obstruction—rare (see Intrahepatic Bile Ducts, under Biliary System, Chap. 20)
- C. Inspissated bile syndrome—probably nonexistent (see "Inspissated Bile Syndrome," under Biliary System, Chap. 20)
- D. Intrahepatic cholestasis from viral hepatitis (see IIIA)
- E. Tumors—rare (see Chaps. 57 and 59)

RELATION BETWEEN SERUM AND TISSUE BILIRUBIN (VISIBLE JAUNDICE)

The relation between serum bilirubin and the tissue discoloration in jaundice is far more complex than would appear on superficial consideration [3638]. The serum level of bilirubin at which tissue jaundice develops and disappears varies in different conditions. With equal bilirubin levels, jaundice appears deeper with impairment of bile flow—in hepatocellular degeneration or cholestasis—than without it—in hemolysis or retention. The excess bilirubin appears to be more easily diffusible when bile flow is impaired. The threshold for the escape of bilirubin into the tissues is often lower than that for excretion in the urine. The rapidity of disappearance of tissue pigment after the serum-bilirubin level drops is greater after relief of extrahepatic obstruction than in hepatocellular jaundice. In general, visible jaundice, particularly of the sclera, appears when the bilirubin level of the serum exceeds 2.0 mg per 100 ml [3638].

BILIRUBIN IN BODY FLUIDS. In the tissue fluids bilirubin is bound to the same protein compound as in the blood. Edema or ascitic fluid and other transudates and exudates contain bilirubin in jaundice; their protein concentration does not clearly influence the bilirubin content [469, 1899]. Concentration of the tissue fluid proteins, as in cutaneous histamine wheals, makes a latent jaundice locally apparent.

Body fluids with low protein content, such as gastric and pancreatic juices, saliva, sweat, and tears [3638], contain as little bilirubin as the tissue fluid. Only in the presence of severe jaundice does the aqueous humor of the eye contain bilirubin, resulting in xanthopsia, or yellow vision.

SPINAL FLUID BILIRUBIN. The plasma-spinal fluid barrier prevents the passage of appreciable amounts of bilirubin into the cerebrospinal fluid except in severe jaundice, which probably injures the barrier in the choroid plexus [65]. If modern sensitive methods are used, very small amounts of

bilirubin can be detected in almost every patient with jaundice [246]. Meningeal inflammation increases the bilirubin in the spinal fluid, as in Weil's disease [496]. Urobilinogen has also been found under abnormal circumstances in the spinal fluid [2407]. In infants, especially those born prematurely, the plasma-spinal fluid barrier is poorly developed, and xanthochromia occurs during the period of icterus neonatorum. The bilirubin in these instances gives an indirect reaction. The cerebrospinal fluid-brain barrier is also poorly developed, and in addition to yellow pigmentation of the dura and choroid plexus, a yellow discoloration of the stem ganglia, including the globus pallidus, thalamus, and the nuclei on the floor of the fourth ventricle and the hippocampus (kernicterus), may be present and may cause serious sequelae (see Kernicterus, under Influence of the Liver on the Brain, Chap. 63).

TISSUE BILIRUBIN. The specific tissue structure to which bilirubin is bound is not fully established, and few chemical examinations are available [3638]. The distribution of the tissue color, especially in jaundice of short duration, suggests that bilirubin is deposited in the elastin of the various organs in addition to the kidney and liver, where it accumulates during excretion. This explains its high concentration in the skin, conjunctiva, nuchal ligament, mucous membranes, and vessels and its absence from lung, myocardium, and muscular tissue [2824]. Little is found in cartilage and less in brain tissue (see Bilirubin, Chap. 11). Injured tissues accumulate bile pigment. Apparently elastin is stained when a certain plasma concentration is reached. A decomposition of the bilirubin-elastin complex may explain the dark-greenish discoloration of the skin in prolonged jaundice. Oxidation processes within the tissues, changing bilirubin to biliverdin, are held responsible for the change of the skin color from reddish yellow to darker green-yellow. In prolonged obstruction, a dark-gray hue is noted and has been called "melasicterus" [1544]. Originally it was assumed that the red hue suggests medical jaundice while a green hue indicates extrahepatic biliary obstruction. This difference, however, is not characteristic enough for differential diagnostic use. Many basic ques-

tions in the distribution of tissue jaundice, such as why jaundice of the trunk usually precedes that of the lower extremities, are still unanswered. The distribution over the body may vary, and partial jaundice has been described. Nervous influences seem to play a role, in that paralyzed extremities may be less jaundiced.

Pseudojaundice. Jaundice may be simulated by the accumulation of substances in blood and tissue which have a yellow color. Some of the substances have different tissue distributions and may fail to stain selectively the elastin of the conjunctiva. Pseudojaundice from accumulation of such substances is usually characterized by white sclerae. The simplest laboratory sign for pseudojaundice is a normal serum bilirubin concentration, as measured by the diazo reaction, in deeply yellow serum, with an elevated icterus index.

CAROTENEMIA. Carotenemia is produced by the accumulation not only of carotene but also of other carotenoid pigments which do not necessarily have biologic vitamin A activity. These pigments are consumed in vegetable foodstuffs. Whether the increase of carotene in the blood is the result of excessive intake of carotenoid-rich food alone, or whether a disturbance in carotene metabolism interferes with the usual disposal of these pigments is unknown. Inability of the liver to transform carotene to vitamin A has been suggested, since carotenemia is found in hepatic disorders. The common occurrence of this condition in diabetes mellitus, where the vitamin A metabolism is known to be altered, supports this hypothesis.

ATABRINEMIA. This condition results from continued use of Atabrine in malarious areas and can easily be recognized by fluorescence of nails. Other less common and usually fleeting instances of pseudojaundice result from the use of picric acid, fluorescein, or acriflavine.

NORMAL COLOR OF SERUM. Under normal circumstances, the yellow color of the serum is accounted for only in part by its bilirubin content. The rest is due to pyrrole compounds such as bilifuscin and possibly xanthorubin, which is found in increased concentration in the hepatectomized dog [3638].

HEPATOCELLULAR DEGENERATION AND NECROSIS: STRUCTURAL ALTERATIONS

Several abnormal processes occur in the liver and biliary tree which are common to a variety of diseases and which are caused by structural and/or functional abnormalities at different sites. They are best discussed as basic units independent of individual diseases, although they are not necessarily a disordered function of one structural hepatic unit.

HEPATOCELLULAR DEGENERATION

The epithelial hepatic cells form the greatest portion of the liver. Therefore, focal or diffuse hepatocellular degeneration produces the most significant functional and structural alterations. These cells appear morphologically simple, yet they perform a great variety of functions, and many severe functional alterations are associated with similar, rather monotonous, structural changes. Hepatocellular degeneration includes cytoplasmic changes frequently associated with secondary nuclear changes, atrophy of cells, and necrosis with disappearance of cells. Functionally, the type of degeneration is less important than its extent. Hepatic-cell degeneration leads to a series of structural and functional consequences, including regeneration (see Regeneration, Chap. 13), inflammation (see Chap. 25), fibrosis (see Chap. 27), and cirrhosis formation (see Chaps. 28, 29). Regeneration, with increased numbers of mitoses

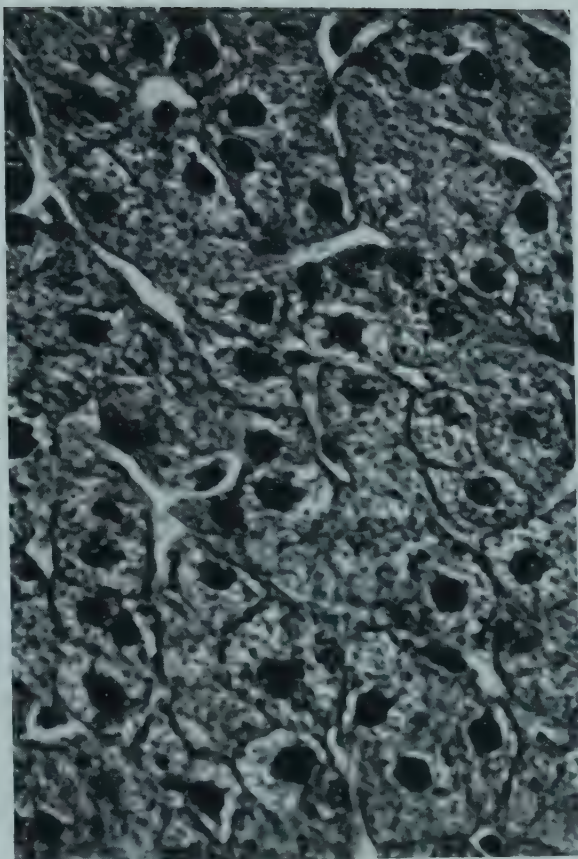
and the formation of two-cell-thick plates, accompanies all forms of hepatocellular degeneration. It may dominate the morphologic appearance, and in biopsy specimens it may be the only indication of existing or preceding hepatocellular degeneration.

Principal Alterations

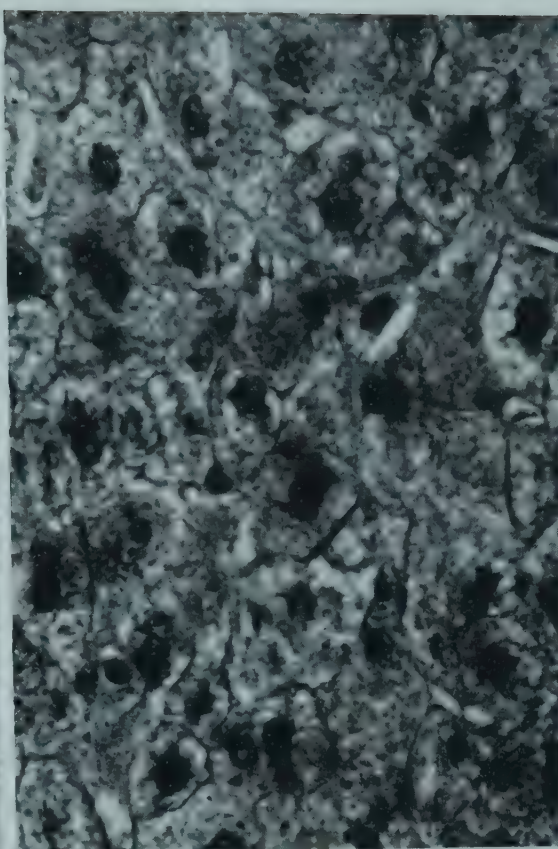
Evaluation of the type and degree of hepatocellular degeneration depends upon the method chosen. Biopsy and autopsy specimens differ considerably in this regard [2625]. In biopsy specimens, a composite picture of degeneration can be recognized and is statistically correlated with the results of hepatic tests, particularly those referring to protein metabolism [2651]. Some variation in the size of the cells and the size of the nuclei in the same lobular zone, as well as a nonuniform appearance of the cytoplasm in the routine section, is evidence of mild hepatic-cell degeneration (Fig. 87). This is in contrast to the normal minor variations of nuclear size in different zones, since the nuclei are larger in the mid-zone than in other parts of the lobule. The mild degrees of hepatic-cell alteration are not necessarily reflected in the results of the hepatic tests in use. Greater degrees of hepatic-cell degeneration are indicated by irregularities of the arrangement of the cells in the hepatic plates. Nuclear alterations include pyknosis, karyolysis, and ballooning. The cytoplasm

FIG. 87 A. Grade 0 hepatic-cell damage. The hepatic cells and their nuclei are of about equal size. They are regularly arranged, and their cytoplasm is uniformly finely granulated and vacuolated, owing to the presence of glycogen. B. Grade 1+ hepatic-cell damage. The hepatic cells and their nuclei vary in size and shape, and their arrangement is less orderly than in A. The cytoplasm also appears less uniform. C. Grade 2+ hepatic-cell damage. The hepatic cells and their nuclei vary greatly in size and shape. The staining quality of the nuclei also varies. The cytoplasm of the hepatic cells appears smudgy and dark in circumscribed areas. In

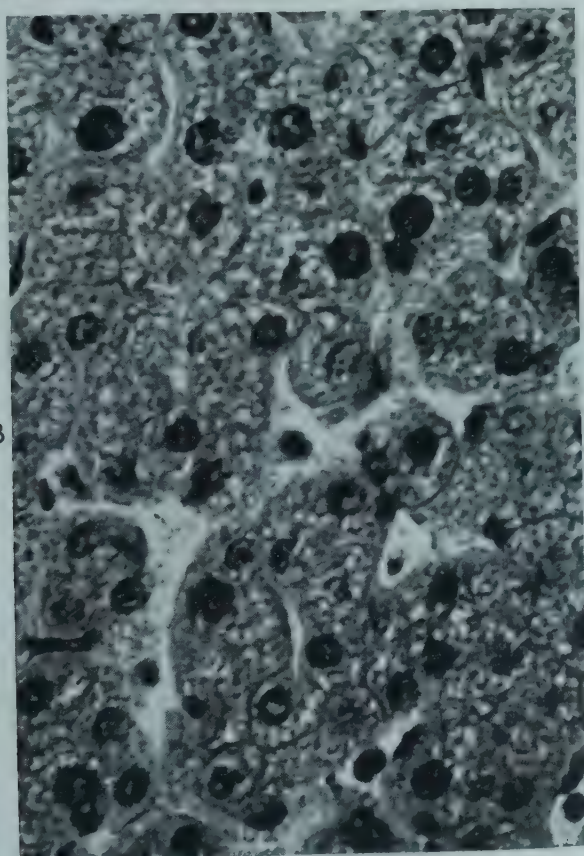
A



C



B



D



others it is ballooned, owing to hydropic degeneration. D. Grade 3+ hepatic-cell damage. The arrangement of the hepatic cells is disorderly. The variations in size, shape, and staining qualities of the hepatic cells and their nuclei are extreme. Many areas of the cytoplasm appear smudgy because of acidophilia. Other areas show hydropic swelling. Nuclear staining is absent in some cells, indicating necrosis. (Popper, H., Steigmann, F., and Szanto, P. B.: *Am.J.Clin.Path.* 19:710, 1949; courtesy of The Williams & Wilkins Company.)

may be arranged in coarse meshes. Basophilic or, more significantly, eosinophilic areas in the cytoplasm cause a smudgy appearance. Clumping indicates beginning coagulation necrosis. The appearance of anuclear cells or cell fragments or areas of frank necrosis implies a more severe degree of hepatocellular degeneration. Necrosis is reflected in widespread homogenization of the acidophilic cytoplasm and coagulation necrosis with loss of nuclear staining.

An attempt to describe specific forms of degeneration would probably be premature and give primitive results. Electron microscopic studies of dry-frozen material may clarify this problem. Some changes result from post-mortem autolysis. Some are different stages in the same process. Advances in histochemistry and cytochemistry have made some differentiation possible, and a few well-defined types of degeneration can be separated from the previously used, all-inclusive entity of parenchymatous degeneration. Atrophy, which is basically a different process, is discussed here because of similar functional consequences. Fat accumulation in the hepatic cell, which is often a degenerative process, is discussed independently (see Chap. 26, Fatty Metamorphosis).

Cytoplasmic Degeneration

Cloudy Swelling. A moderate enlargement of the liver occurs in many conditions in which an injury to the liver is part of generalized tissue damage; for instance, in infections, intoxications, and so-called "toxemias." Various terms have been applied to this condition. The term "parenchymatous degeneration" refers to the histologic changes, and "cloudy swelling" refers to the cooked appearance of the liver.

Grossly, the liver is larger than normal; its edge is rounded; its consistency is reduced; it bulges on the cut surface, and its lobular markings are somewhat obscured. The actual histologic, histochemical, and microchemical changes of this common condition are still debated, particularly in the German literature, where great emphasis is placed on it [894]. Many of the changes may be post-mortal, since similar alterations are observed as the result of postmortal autolysis. Thus part of the phenomenon has been interpreted as an exaggeration of postmortal autolysis resulting from a premortal hepatic injury [1365]. In liver biopsy specimens, parenchymatous degeneration is not clearly discerned and the diagnosis should not be used.

In autopsy specimens, the hepatic cells are swollen and the cytoplasm is granular, supposedly because of protein precipitation, giving rise to the term "albuminous degeneration." Small vacuoles apparently containing fluid, may be present. The cytoplasm appears darker and less vacuolated than normal as the result of a decrease in glycogen. It is sharply differentiated from the lining of the bile canaliculi. Cytoplasmic basophilia is sometimes reduced. The mitochondria appear swollen [3727], and swelling of cytochondria, mitochondria with a nucleic acid shell, has been considered the cause of some cytoplasmic granularity [2489]. The lesion supposedly signifies increased hepatic activity, an anabiotic process [1962]. The chemical basis of the process is not clear. Abnormal acidification has been suggested [1697]. Increased protein or amino acid content [894] and disturbances of enzymatic function of the hepatic cell [1292] have been claimed. Exposure of hepatic cells in vitro to hypotonic solutions produces changes similar to cloudy swelling, such as impaired mitochondrial staining and the formation of foamy cytoplasm [2489]. Probably the best explanation is that it results from a disturbed permeability of the hepatic-cell wall, with imbibition of the cell by serum proteins and by hydrating sodium ions [944]. In conclusion, the lesion seems to develop primarily in the period immediately before death, and its functional significance appears minimal.

Acidophilic Degeneration. If the normal basophilia of the hepatic-cell cytoplasm, mainly from pentose nucleoprotein, disappears, acidophilic protein predominates [3287]. In sections stained with hematoxylin-eosin, this change is characterized by a bright-pink appearance—acidophilic or eosinophilic degeneration. This lesion precedes nuclear changes and is the first morphologically recognizable response of the hepatic cell to injury [752, 1898, 2489, 2626, 3287]. It also occurs as a result of nutritional deficiency. Loss of basophilia is usually reversible and is not the sole phenomenon responsible for eosinophilic degeneration. An increase of acid valences is suggested by histochemical studies [3614]. The intravital uptake of acid azo dyes is also intensified and is seen as a diffuse cytoplasmic stain [3613].

Cytoplasmic Coagulation. Clumping of the cytoplasm associated with an acidophilic reaction—the protein is an advanced stage of acidophilic degeneration. Such acidophilic clumping of the cytoplasm, termed "hyalinization," occurs in he-

patic cells in many different conditions. It depends largely on the type of fixative.

MALLORY BODIES. Ramified bodies, seen around the nucleus, seem to have developed from granules which have coalesced. They are conspicuous in cells with otherwise fatty or hydropic cytoplasm (Fig. 88C). They are more easily demonstrable with special stains [1979]. Mallory considered them characteristic for alcoholic cirrhosis [2187]. They are found, however, in many other types of hepatic injury.

ACIDOPHILIC BODIES. Granular coagulation differs from diffuse homogeneous acidophilia, which starts in some portion of the cytoplasm and eventually involves the entire cell [215]. The nucleus first becomes pyknotic and subsequently disappears, at which time the cell is expelled from the hepatic-cell plate into the tissue space as a globular, very refractile body. Such acidophilic bodies were described by Councilman in yellow fever, where they may be pigmented [3425]. A similar finding has, however, been considered characteristic for human viral hepatitis [125, 1715, 2189, 3541], especially in the acute stage (Fig. 88A and B). They are also found in infectious mononucleosis [3452] and in canine viral hepatitis [2847]. This diffuse acidophilic coagulation, which has been considered to harbor the virus [213], is not specific for viral diseases, however, since it has also been found after burns treated with tannic acid [210] and in bromobenzene intoxication [1822].

Hydropic Degeneration. **MORPHOLOGY.** Isolated cells or groups of hepatic cells may show a rarefaction of the cytoplasm, usually associated with a central position of the nucleus and sharply defined cellular borders. The cytoplasm appears vacuolated with routine stains, but the vacuoles contain neither fat nor glycogen, and little or no acidophilic material is noted. If present, it takes the form of a fine film. The material which displaces the cytoplasm appears to be aqueous, justifying the term "hydropic degeneration." In man, a vacuolar and a plantlike cell pattern of reaction have been differentiated [1172]. In the former, the vacuoles are found anywhere in the cell. They vary in size but do not fill the entire cytoplasm. If they are large, they either indent the nucleus or are indented by it. In the plantlike cell form, the cytoplasm is rarefied, the cell is larger, and only a few eosinophilic or basophilic threads accumulate on the periphery and seem to suspend the nucleus (Fig. 88E). In the presence of jaun-

dice, in the wide rarefied zone between the cell border and nucleus, distinct, usually bile-pigmented granules are noted. This results in isolated cells, with rarefied ballooned cytoplasm, which are found in various types of human hepatic injury, usually in the center of the lobule. In viral hepatitis, the "balloon" cells are at least twice as large as normal cells. They have a small nucleus surrounded by a narrow zone of condensed cytoplasm (Fig. 88D). Similar cells occur in prolonged extrahepatic cholestasis. Hydropic degeneration can proceed to necrosis of the cell but is apparently reversible. The cell is not necessarily functionally paralyzed, as judged from the persistence of glycogen, mitochondria, or basophilic granules in the sparse cytoplasm [3361], although in human liver biopsy specimens cytoplasmic basophilia is usually absent [3287].

HISTOCHEMISTRY. The actual histochemical nature of the process and the type of protoplasmic alteration are still unsettled [55, 3361]. Increased permeability, permitting the invasion of the cells by tissue fluid or serum [55], still appears to be most plausible. The protein material within the vacuoles is possibly fibrin [3361].

ETIOLOGY. In experimental intoxications, such as carbon tetrachloride [467], chloroform [1894], and bromobenzene [1822] poisoning, large hepatic cells are found chiefly in the centrolobular zone. The cytoplasm appears almost colorless, and the nuclei are usually centrally placed. The most clearly understood example is the lesion in man and animals which results from anoxia. It is seen in animals [55, 2591, 3361] and in aviators exposed to low atmospheric pressure [1868]. The vacuoles in the anoxic lesion are surrounded by a condensation of the cytoplasm and contain sharply defined spherules and rodlets [55, 1172, 3361]. The same lesion also occurs in other conditions associated with anoxia [1172], and it may appear even in the absence of general anoxia as a result of local oxygen want. It may also be caused by enzymatic disturbances which produce local respiratory deficiency [55]. Sometimes hydropic cells, isolated or in groups, are present without any obvious reason.

Feathery Degeneration. In prolonged intrahepatic or extrahepatic cholestasis, hepatic cells laden with bile pigment or near bile plugs have a rarefied cytoplasm, in which a fine brown pigmented protoplasmic network is found. This has been called "feathery degeneration" [3549] (Fig. 88F). The nucleus is central and pyknotic. The

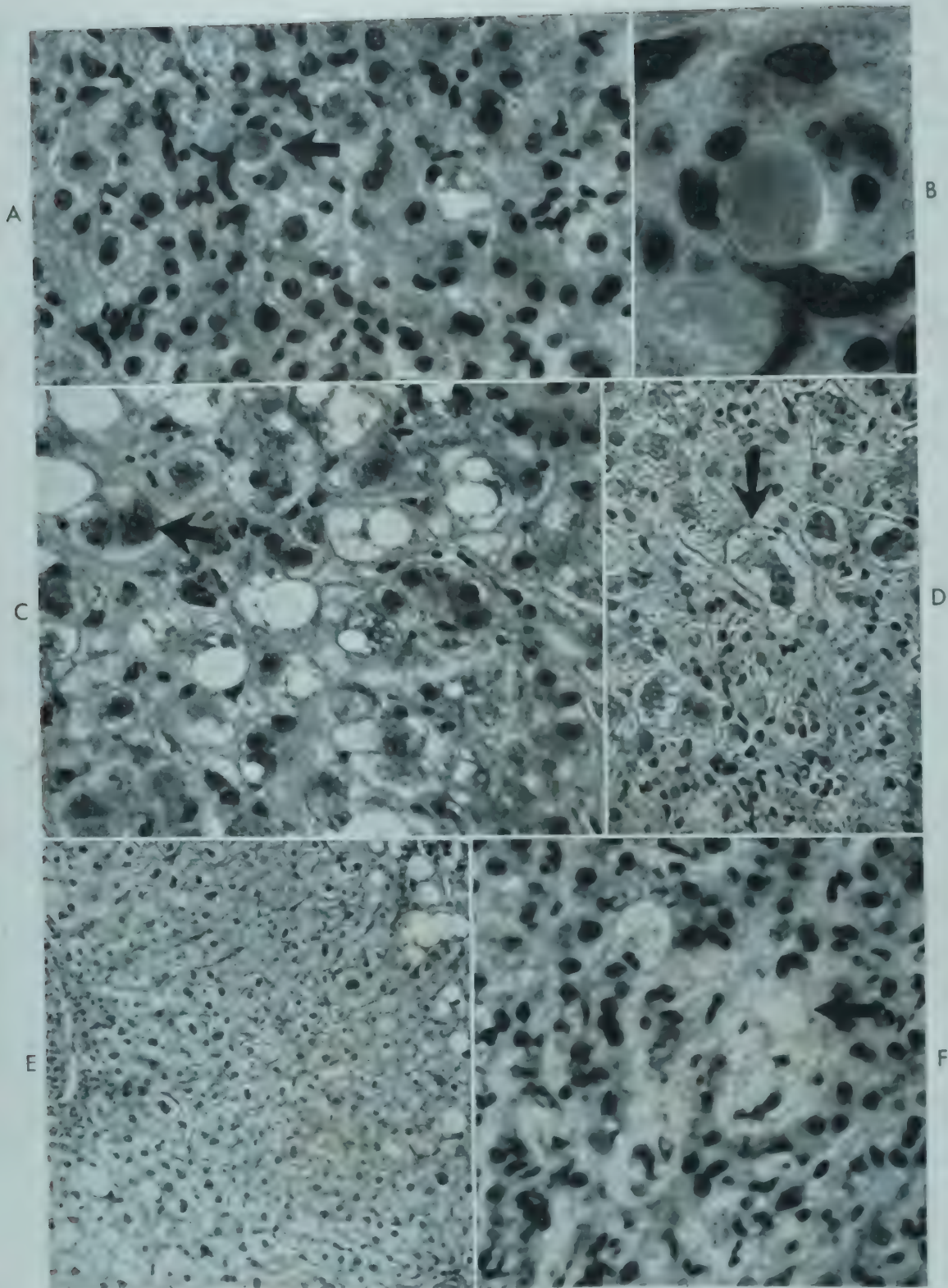


FIG. 88 A. Cell with homogeneously acidophilic cytoplasm expelled from the hepatic-cell plate in viral hepatitis. H&E ($\times 315$). B. Higher power of same ($\times 630$). C. Coagulated acidophilic clumps in the cytoplasm ("Mallory bodies") in an alcoholic patient with florid cirrhosis. H&E ($\times 315$). D. Ballooning of hepatic cells containing bile-pigmented granules (hydropic swelling) in viral hepatitis. H&E ($\times 220$). E. Plant-cell-like appearance of hydropic hepatic cells in a patient with postnecrotic cirrhosis. H&E ($\times 105$). F. Irregular rarefaction of the cytoplasm of hepatic cells around large bile plugs in cholestasis due to extrahepatic biliary obstruction. H&E ($\times 315$).

changes involve scattered cells, mainly in the center of the lobule, and groups of cells on the periphery of the lobule in bile infarcts [2154] (see Hepatic-cell Necrosis, under Morphologic Appearance, in Chap. 24).

Atrophy

Atrophy of the hepatic cells may be of two types: pressure atrophy with compression of hepatic cells, or primary atrophy resulting from generalized or local tissue undernutrition.

Internal Pressure. Pressure atrophy results from space-occupying processes, either in the form of focal lesions such as tumors or abscesses exerting pressure on the surrounding tissues, or in the form of a widening of the structures adjacent to the hepatic-cell plates as in amyloidosis or congestion. In either instance the hepatic cells become flat, and the plates thin (Fig. 89B). The basophilic hue of the cytoplasm does not decrease and may even increase, at least in the earlier stages. These pressure effects do not necessarily interfere with the function of the cell [3287]. With severe generalized compression of the hepatic-cell plates, as in amyloidosis, the basophilia disappears [3287], and functional impairment results. Eventually the hepatic cells disappear.

External Pressure. Occasionally, circumscribed depressions, up to several centimeters in diameter, are seen on the surface of the liver. On the base of these, hepatic-cell plates are lost and the framework is collapsed, with some ductular proliferation in the ghost lobules. The picture resembles focal massive or sometimes submassive collapse following massive necrosis. In this area, partial endophlebitic obstruction of portal and hepatic vein branches occurs. These depressed areas usually coincide with rib or corset pressures (see Rib Impressions, and Costal Arch Grooves, under Malformations and Malpositions, Chap. 20) and are therefore probably the result of pressure atrophy rather than the result of focal disease.

Atrophy from Undernutrition. LOCAL ATROPHY. Interruption of a branch of the hepatic duct or portal vein results in atrophy of the part of the liver supplied by the duct or vein. This is usually associated with hypertrophy of the remaining part of the liver [2211]. While atrophy following ligation of the duct is probably a pressure effect from the distended biliary passages, that following interruption of the blood flow is an example of a local nutritional disturbance. In man, such disturbances of tissue nutrition are responsible for

atrophy of the left lobe of the liver. This occurs as a morphologic entity with little clinical significance [225]. The left lobe appears shrunken, with its capsule wrinkled and thickened. Histologically, the lobules are greatly reduced in size, and the portal tracts and central fields are approximated. Atrophy of the left lobe is usually caused by obstruction of portal vein or bile duct branches or by echinococcal cysts [1572], although in some cases no cause is apparent.

BROWN ATROPHY. In various conditions with depression of the metabolic processes, such as old age, starvation, and carcinomatosis, the entire liver and each lobule become smaller, and wear and tear pigment is deposited, mainly in the center of the lobule. The liver appears brown, and its edges are sharp. It is firmer than normal, and the lobular markings are hazy. The basophilia is increased, probably owing to increased concentration in the smaller cells. The cells, which contain much wear and tear pigment, may show a decrease in basophilia [3287] (Fig. 89A) (see Hepatic Brown Atrophy from Undernutrition, Chap. 51). The brown atrophic liver shows little functional impairment.

NECROSIS

Necrosis is characterized by the presence of cell fragments or dead hepatic cells without nuclear staining, or by the absence of the cells with inflammatory cell response, collapse, or red cell engorgement of the framework. It is an advanced and irreversible stage of degeneration. Whether hepatic-cell remnants are found or not depends upon the duration of the lesion, as well as upon the type of necrosis. For instance, in most chemical injuries, the cells die slowly and attract segmented leukocytes, whereas in viral diseases the cells usually disintegrate into small fragments, which attract histiocytes [2632] (see under next heading, Focal Necrosis). The reticulum and collagen framework does not become necrotic unless ischemia is present. Necrosis can be classified according to its localization and its extent (Fig. 90).

Focal Necrosis. Focal necrosis is the death of one cell or a small group of cells anywhere in the lobule. The term is usually applied to designate circumscribed foci in which the hepatic cells have become necrotic and in many cases have disappeared (Fig. 89D). Segmented leukocytes and histiocytes, with some proliferation of the Kupffer

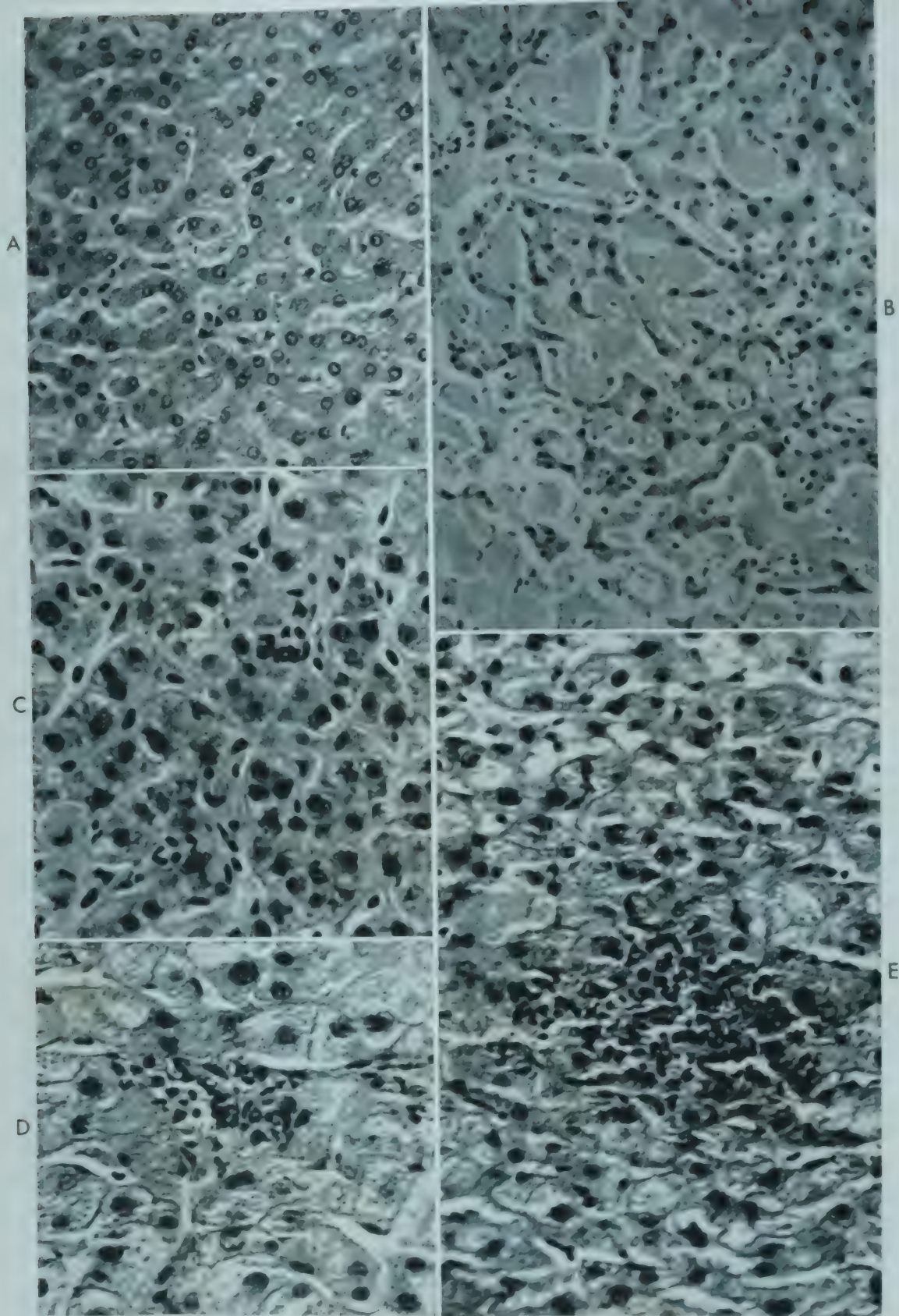


FIG. 89 A. Brown atrophy with thinning of hepatic-cell plates in carcinoma of the esophagus. The cytoplasm appears homogeneous. H&E ($\times 170$). B. Amyloid deposits compressing hepatic cells in chronic pulmonary tuberculosis. H&E ($\times 125$). C. "Spotty" necrosis (single cell necrosis) in acute viral hepatitis. Accumulation of round cells in place of hepatic cells that have disappeared. The surrounding parenchyma shows hepatic-cell degeneration and great variation from cell to cell. H&E ($\times 315$). D. Focal necrosis in nonspecific reactive hepatitis caused by tuberculosis. Hepatic-cell remnants are surrounded by segmented leukocytes. The surrounding hepatic cells are normal. H&E ($\times 270$). E. Extensive focal necrosis in lobar pneumonia, with accumulation of segmented leukocytes. Surrounding hepatic cells show severe damage. H&E ($\times 270$). (D and C, Popper, H.: *Am.J.Med.* 16:98, 1954.)

cells, may be the only indication of the lesion. Small fragments of hepatic cells and bacteria are occasionally recognized. The foci vary in size from loss of one or a few cells to areas 1 mm in diameter, the larger ones usually being located in the intermediary zone of the lobule (Fig. 89E). The pathogenesis is either local cell death caused by bacterial toxins or by obstruction of sinusoids by proliferated Kupffer cells or fibrin thrombi [2187, 2632]. The circumscribed necrotic foci in diphtheria are an example of a toxic injury which may become quite extensive. Vascular obstruction has been demonstrated in the typhoid nodule in the liver [2187], but it does not explain most instances, and the presence of cytolytic enzymes has been claimed [1697]. Focal or single-cell necrosis

develops rapidly, appearing, for example, during the course of a surgical operation, as demonstrated by multiple biopsies [1714].

The cellular response depends upon the type of injury. In viral infections, in contrast to bacterial infections, monocytic elements predominate and segmented leukocytes are rarely seen in small foci (Fig. 87C). Focal necrosis may be the precursor of necrotizing or granulomatous foci in brucellosis, tularemia, miliary tuberculosis, sarcoidosis, and syphilis. Functionally, the lesion is not associated with alterations in any of the hepatic tests, even if it is widespread [1074]. If functional hepatic alterations are noted, they are caused by associated diffuse changes in the hepatic parenchyma.

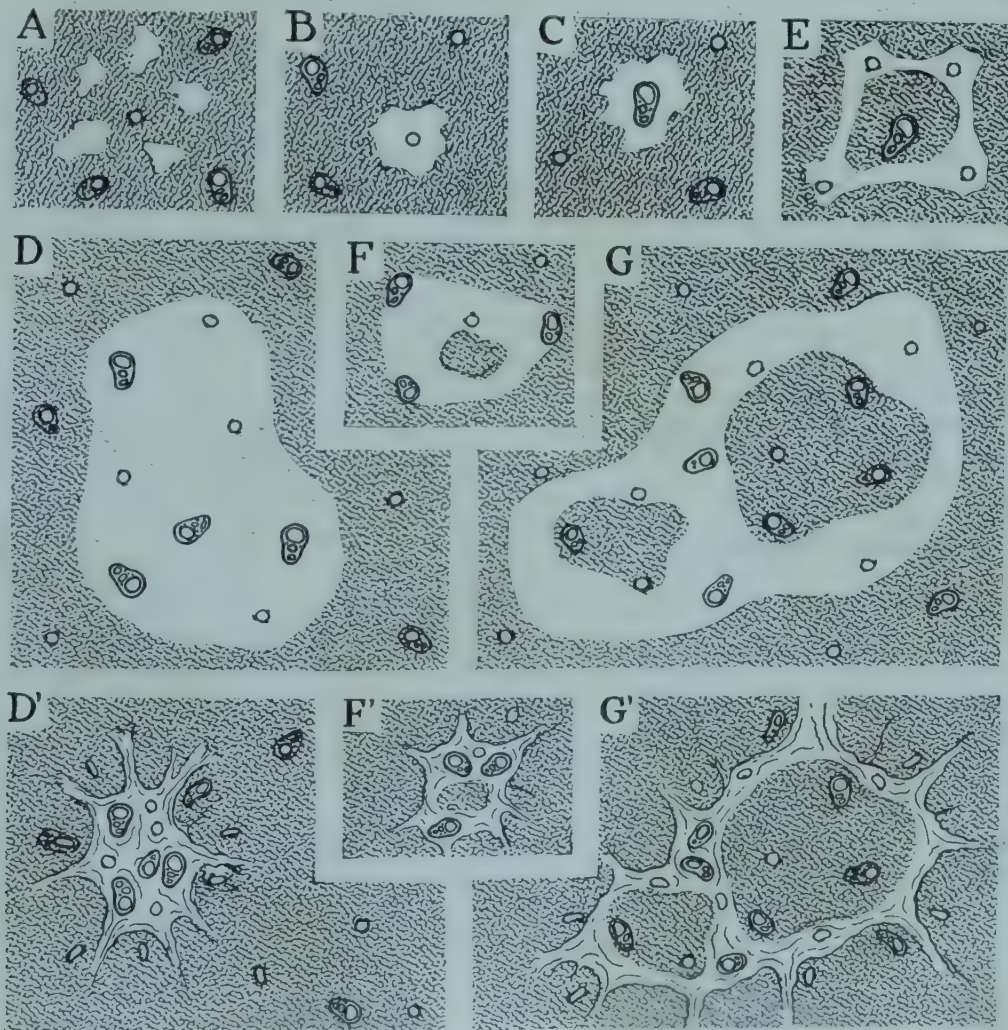


FIG. 90 Diagram of types of hepatic necrosis and collapse. Portal tracts are represented by heavy circles, and central veins by light circles. A. Focal necrosis. B. Central necrosis. C. Periportal necrosis. D. Massive necrosis with subsequent collapse (D'). E. Central bridging of areas of necrosis. F. Submassive necrosis with a fragment of one lobule remaining, with subsequent collapse (F'). G. Submassive necrosis with multinodular fragments becoming nodules in subsequent collapse (G'). (Redrawn from Popper, H.: *Am.J.Med.* 16:98, 1954.)

Zonal Necrosis. Necrosis may involve an entire zone of the lobule, possibly as an expression of a specific function or vulnerability of that zone.

CENTRAL NECROSIS. Degenerative, or necrobiotic, changes and disappearance of epithelial cells from the centrolobular portion are commonly found; more often in autopsy material than in biopsy specimens (see Central Necrosis, under Agonal and Postmortal Changes, Chap. 20). It is readily produced by various poisons in experimental animals such as the rat, in which it is fully developed in less than 48 hours.

Macroscopic Appearance. The lobular architecture of the liver is usually exaggerated, in that the central zones are widened, dark red, and sunken. Sometimes they are connected by red bridges of similarly sunken parenchyma. The lobular pattern appears reversed, because the intact periportal area is surrounded by a red hemorrhagic or necrotic zone (Fig. 91A).

Histologic Findings. If hepatic cells are present, their cytoplasm is eosinophilic and shows irregular vacuolization, granulation, and foci of hyaline necrosis. Nuclear staining may be absent, and the cells may be fragmented (Fig. 91B). Edema distends the perisinusoidal spaces, and the sinusoids are narrow and may contain fibrin threads. Subsequently, the hepatic cells and their fragments disappear and are replaced by red cells occurring within or outside the dilated sinusoids. Segmented leukocytes occasionally aggregate around the disappearing cells. The Kupffer cells are usually proliferated, and some histiocytic infiltration may be noted. The histiocytes and Kupffer cells contain lipofuscin pigment. Finally, the framework collapses in the central zone (Fig. 91C). The ductules are often increased and sometimes dilated. The portal tracts are infiltrated in the presence of extensive lesions. The extent of the necrosis is usually uniform throughout the same liver. It can involve the medial two-thirds of the lobule and even reach to the portal tracts. The central portions of neighboring lobules may become connected by bridges of necrosis, which produce the grossly visible network of red and sunken areas on the cut surface. Central necrosis is sometimes modified by the presence of fat in the intermediate zone bordering the area of necrosis, as found in carbon tetrachloride intoxication [2187, 2336].

Etiology. Experimentally, central necrosis is produced in its purest form by prolonged exposure to low atmospheric pressure [55]. Intoxications

with bromobenzene or carbon tetrachloride, for instance, cause central necrosis (see Central Necrosis with Fatty Metamorphosis, Chap. 41), as does experimental passive congestion. In man, perfusion of the liver with peripheral venous blood [2175], acute reduction of hepatic vein flow, and suppression of hepatic arterial flow [1236, 1497] produce it. Central necrosis is very common found in autopsy specimens, especially in various infections or chemical intoxications. It is also associated with endogenous processes such as hyperthyroidism, uremia, pulmonary embolism, and myocardial infarction, the common denominator of which is passive congestion [2128, 2187, 3470]. It is seen in uncomplicated passive congestion [2187]. It also occurs in shock [2177, 2337] and following hepatic artery ligation [2220].

"Toxic" vs. "Congestive" Necrosis. In most instances, differentiation of centrolobular atrophy or hepatic cells caused by congestion from primary necrosis caused by toxins, both leading to disappearance of cells, is difficult, since "congestive" and "toxic" factors are often intermixed. Analysis of relatively pure examples of each type, however, reveals characteristic differences [3470]. Congestion is typified by large central veins with wide or normally open branches piercing through the walls, disappearance or atrophy of hepatic cells with no fragments remaining, and dilatation of the sinusoids (Fig. 91D). In toxic necrosis, nuclear fragments surrounded by exudative cells are noted. Despite the fact that the sinusoids are frequently dilated, the central veins and their piercing branches are relatively narrow (Fig. 91B). In both instances the localization of the lesion in the central portion is considered to be the result of circulatory disturbances. In the congested form the circulatory disturbance is caused by heart failure, whereas in toxic necrosis it is caused by contraction of the central vein or of the hepatic vein tree [2175, 3470] and by swelling of the hepatic cells in the peripheral portion of the lobule [1193, 1497]. Shock may also play a role.

Functional Correlation. The infrequency with which central necrosis is encountered in biopsy specimens suggests that circulatory disturbances in the agonal period are responsible for its frequency in autopsy specimens [2625] (see Central Necrosis, under Agonal and Postmortal Changes, Chap. 20). In congestion little correlation exists between histologic appearance and hepatic function, Bromsulphalein retention being the

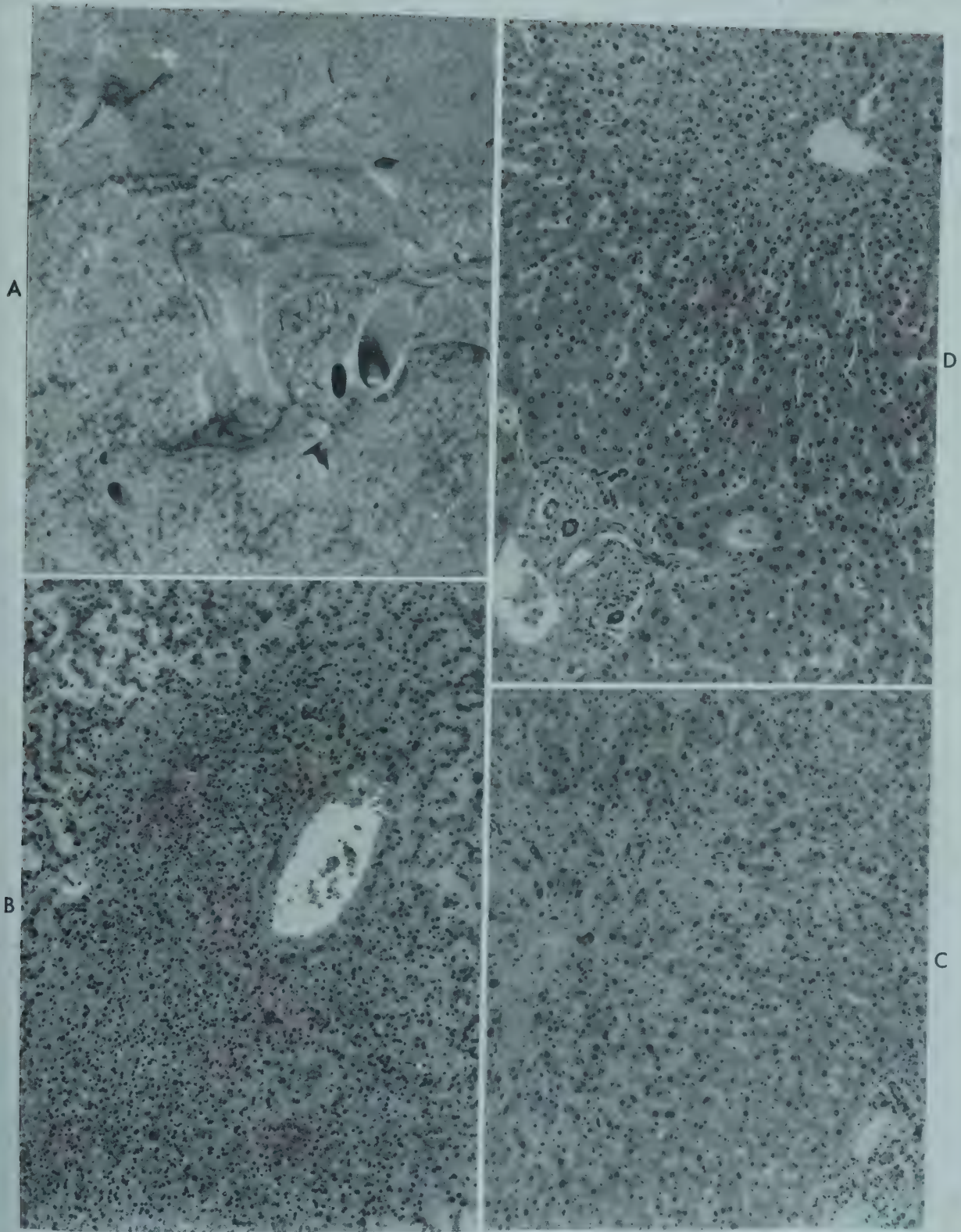


FIG. 91 *A.* Cut surface of liver of patient with tuberculous peritonitis revealing central necrosis represented by sunken red areas. In some areas, red bridges connect adjacent central zones, thus reversing the lobular architecture. *B.* Necrobiotic cells and nuclear cell fragments, as well as exudative cells surrounding a sublobular vein. H&E ($\times 80$). *C.* Central necrosis with disappearance of necrotic cells and collapse of the framework. H&E ($\times 110$). *D.* Central congestive necrosis, with red cells replacing the missing hepatic cells. H&E ($\times 65$).

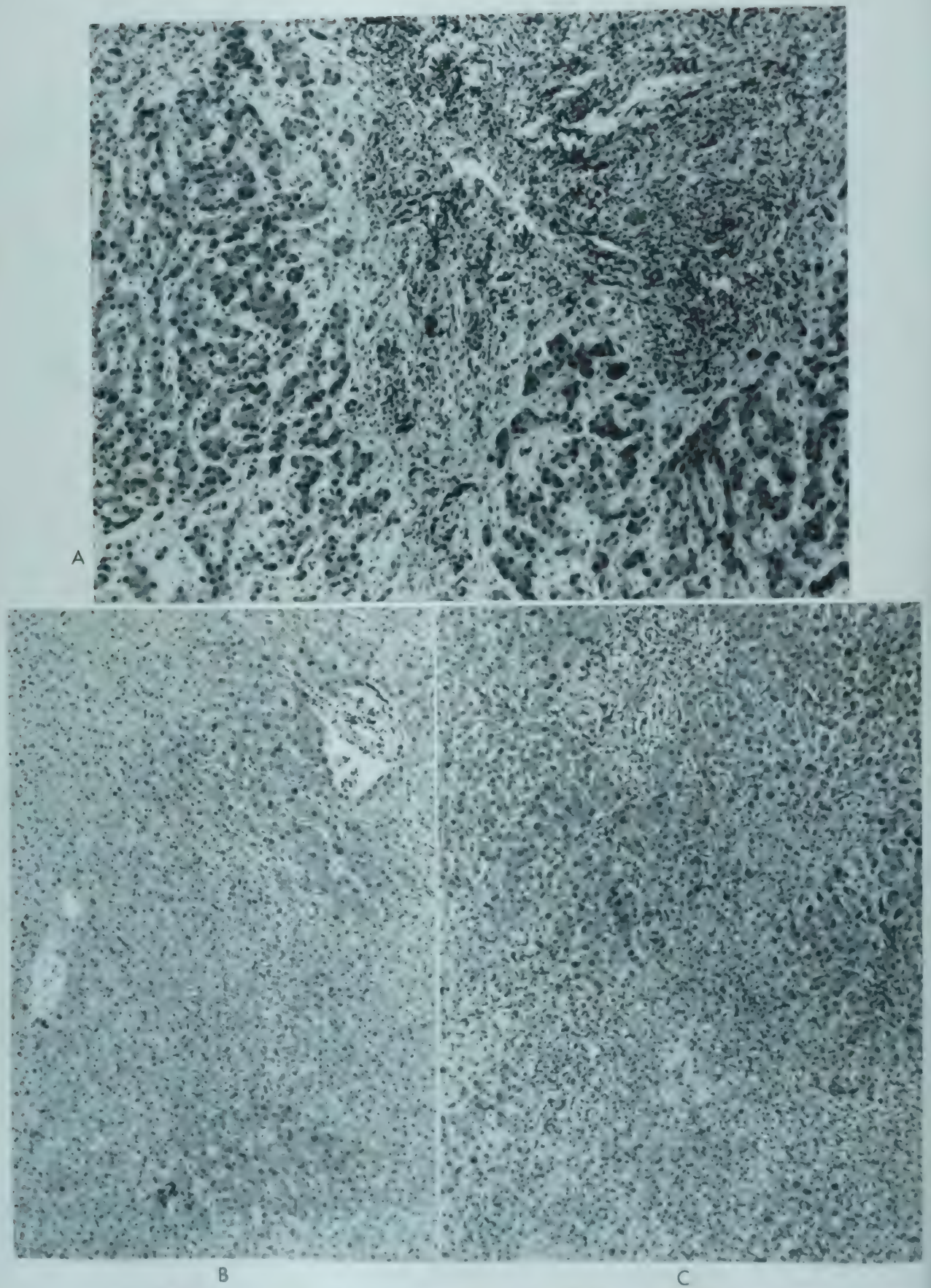


FIG. 92. A. Peripheral necrosis in infected biliary hepatitis. H&E ($\times 85$). B. Extensive central necrosis, with necrotic bridges between intact periportal zones, in toxic hepatic injury in lobar pneumonia. H&E ($\times 65$). [Poppel, H.: *Am J Med*, 16:98, 1954.] C. Submassive ischemic necrosis following traumatic injury to the liver and extending to the portal tract. A few segmented leukocytes are seen in the intact border zone. H&E ($\times 70$).

conspicuous abnormality. In toxic necrosis, functional impairment may be extensive, possibly owing to diffuse hepatocellular involvement.

MID-ZONAL NECROSIS. Mid-zonal necrosis with intact central and peripheral zones is rare in human pathology. It occurs in yellow fever and has been described following trauma [459], although under these circumstances the central zone is also involved. In passive congestion, especially in young people, mid-zonal necrosis develops around a central accumulation of fat. In animal experiments mid-zonal necrosis has occasionally been observed in burns [210], but it is found most consistently after administration of chloroform to hyperthyroid rabbits [294].

PERIPHERAL NECROSIS. Experimentally, necrosis has been produced exclusively on the periphery of the lobule by intravenous injection of chloroform or by allyl formate intoxication [945, 2831]. Peripheral necrosis is also produced by simultaneous injection of oil into branches of the hepatic artery and portal vein, while injection of one vessel produces central necrosis [1236]. Reversal of the blood flow by obstruction of both vessels supposedly renders the peripheral zone the farthest removed from oxygenated blood and thus makes it most vulnerable to anoxia. In man, phosphorus intoxication is often listed as a cause of peripheral necrosis, but there is no recent confirmation for this. Peripheral necrosis has been described in infants with ferrous sulfate poisoning [2090A]. The changes in eclampsia are also listed with this group, although the lesion is not strictly peripheral (see Hepatic Necrosis in Eclampsia, Chap. 49). The most common form of peripheral or periportal necrosis is associated with and possibly caused by inflammation on the periphery of the lobules and in the portal tracts. The same lesion occurs in cirrhosis on the border between parenchyma and septums. In this sense peripheral or periportal necrosis is part of periportal inflammation (see Periportal Inflammation, under Focal Necrosis, Chap. 25). In the area of inflammation, the limiting plate is destroyed and replaced by inflammatory and scavenger cells, many being segmented leukocytes (Fig. 92A). This lesion is therefore found in many hepatic conditions associated with intralobular necrosis, such as viral hepatitis, and also in portal inflammation in extrahepatic cholestasis. In the healing stage, the limiting plate is re-formed on the border of the surviving hepatic cells, thereby increasing the size of the portal tract [2463].

Massive and Submassive Necrosis. **SUBMASSIVE NECROSIS.** Frequently, necrosis of the lobular center progresses, with the formation of bridges of necrotic tissue between neighboring lobules (Fig. 92B). The necrosis may extend further in some areas, and, in submassive necrosis, it reaches the periphery of the lobule [3316] (Fig. 92C). Irregular islands of intact tissue remain, varying in size from part of one lobule or garland-shaped parts of adjoining lobules to large multilobular remnants. Necrotic areas subsequently collapse, creating stresses on the remaining fragments. Fissures develop, along which stretched and disintegrated hepatic cells are noted (Fig. 90) (see Fibrosis Following Collapse, under Forms of Fibrosis, Chap. 27). This leads to further subdivision of the surviving fragments. In the smaller remnants particularly, the hepatic cells are rearranged by the alteration of the sinusoidal blood flow [2630]. Moreover, regeneration within and on the border of the remaining tissue rounds out these areas to form nodules (see Formation of Regenerative Nodules or Pseudolobules, under Processes Common to All Types of Cirrhosis, in Chap. 28).

MASSIVE NECROSIS. Massive necrosis is the disappearance of all hepatic cells in the lobule (Fig. 93, top). Some hepatic cells may persist as ghost cells without nuclear staining or as smaller fragments. In early stages, proliferated Kupffer cells and exudate cells engaged in phagocytosis intermixed with red cells within and outside the sinusoids occupy more space than the intact hepatic-cell plates. In later stages, the excess blood cells and the exudate cells disappear, so that the lobule shrinks considerably in size. The reticulum framework is intact, although it is distended early and collapsed in later stages. The shrunken lobule, still outlined by the distribution of the approximated hepatic and portal canals, has become a ghost lobule following the massive collapse (Fig. 93, bottom). The portal tracts are identified by appearing more dense than the collapsed framework, in sections prepared with connective tissue stains, and by their infiltration with cells. Massive necrosis may involve only isolated lobules, or it may occur diffusely throughout the entire liver or large parts of it. With diffuse involvement the liver is still of normal size in the very early stage, and the lobular architecture is exaggerated on the cut surface. With the disappearance of cells and fragments, the liver shrinks. The anterior edge becomes sharp, and the consistency is reduced to such a degree that the organ can be bent at will.

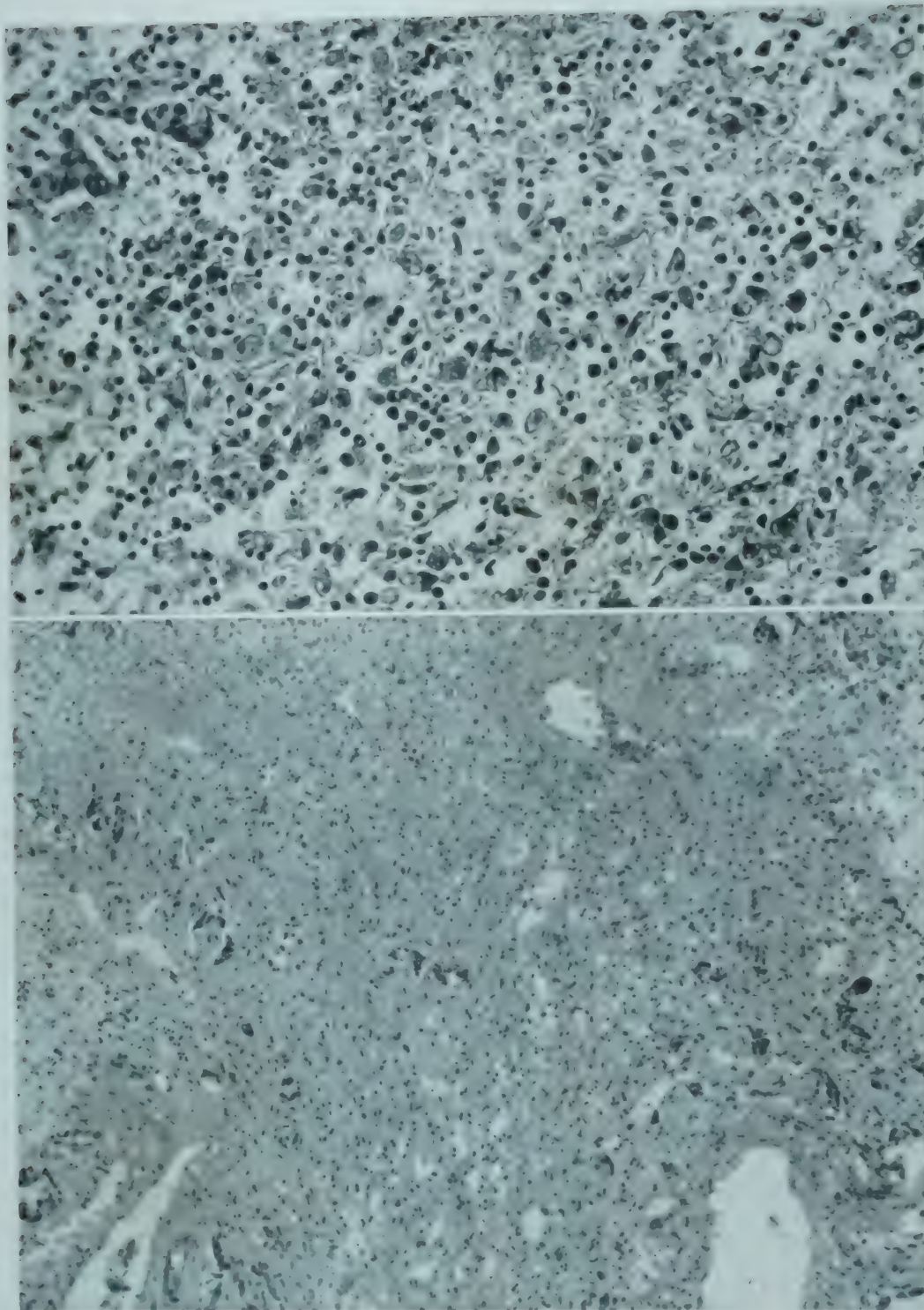


FIG. 93 *Upper.* Massive necrosis of hepatic cells, with accumulation of scavenger cells and a few proliferating ductules, in fulminant hepatitis. H&E ($\times 160$). *Lower.* Recent massive collapse of framework after disappearance of necrotic hepatic cells and accompanying exudate in acute viral hepatitis, with approximation of central and portal fields and proliferation of ductules. H&E ($\times 82$).

"Acute Yellow or Red Atrophy." If the disintegrating hepatic cells are rich in fat, the color is yellow. If fat is not prominent, as is usually the case, a brown-red color is seen. Nevertheless, the term "acute yellow atrophy" has been coined for this. The term "red atrophy" has been applied in the later stages, when the hepatic cells are gone and the color of the erythrocytes predominates in

a shrunken liver which looks like a spleen on the cut surface. At present, better knowledge of the etiology of massive necrosis has resulted in replacement of these time-honored, purely descriptive terms.

Etiology. Although massive and submassive necrosis result from progression of focal or zonal necrosis, the rapidity of the progression usually pre-

vents the recognition of an original zonal lesion. Human massive necrosis is caused by various poisons, but more commonly it occurs in infectious hepatitis (see Massive Necrosis, under Structural Alterations, in Chap. 43). A morphologic difference between toxic and viral forms of massive necrosis has been suggested [2632]. In animal experiments, massive necrosis occurs as a result of dietary deficiencies, especially of sulfur amino acids (see Nutritional Hepatic Injury in Animals, Chap. 50) [1320, 1498, 2968]. This has been considered to be the result of a swelling of the peripheral hepatic cells, with subsequent obstruction of the sinusoidal lumen in the rest of the lobule, resulting in death of the cells [1497]. Whatever the etiology of massive necrosis, the characteristic end result is that the collapsed area can not reexpand.

Anoxic Necrosis. The hypoxia in zonal, massive, or submassive necrosis usually does not injure the less sensitive framework or the Kupffer cells and other mesenchymal cells (Fig. 92C). These cells become necrotic only with complete anoxia, and when the framework ruptures, at least in some areas. This occurs near traumatized surfaces, after ligation of the hepatic artery, after involvement of intrahepatic arterial branches in periarteritis nodosa or malignant hypertension [2349], and in systemic infarcts. A rim of leukocytes often demarcates the necrotic zone, in which no nuclear staining is visible.

Chemical Changes in the Liver. Chemical changes usually result from the opposing processes of destruction and regeneration [3241, 3367]. Some of the changes depend on the type of injury, but other alterations are seen in all types regardless of etiology.

INCREASED WATER CONTENT. In most types of hepatic injury, the water content of the cell is increased [1497]. This is noted in nutritional necrosis, as well as in carbon tetrachloride necrosis [3367]. This increase in intracellular water makes changes in concentrations of solids difficult to evaluate.

GLYCOGEN DISAPPEARANCE. One of the earliest changes noted histologically and chemically is the disappearance of glycogen [334, 2652]. Hepatic glycogen depletion is probably the oldest observation concerning chemical alterations associated with hepatic injury. In the recovery period or in subsequent cirrhosis or hepatoma formation, the glycogen content increases [3241], possibly owing to reduced glycogenolysis [2496].

NITROGENOUS SUBSTANCES. The protein content of the liver is reduced [477, 1823]. In dietary injury and in milder degrees of human hepatic injury (see Cloudy Swelling, under Cytoplasmic Degeneration, earlier in this chapter), protein seems to increase [894, 1498, 3385]. An increase in nonprotein nitrogen occurs in chloroform and phosphorus poisoning but is not found in dietary injury [1498]. The reduction in pentose nucleic acids suggested by the disappearance of the cytoplasmic basophilia in all types of human [2131, 3287] or experimental hepatocellular degeneration is not necessarily apparent by chemical analysis if the initial body weight is taken as a reference point [477, 976].

LIPIDS. The fat content of the liver is increased in some hepatic injuries, such as in carbon tetrachloride intoxication (see Acute Carbon Tetrachloride Intoxication, under Experimental Hepatic Injury, Chap. 41). This increase does not occur in other types, such as dietary necrosis [1498] or chronic ethionine intoxication [1825]. The phospholipid content shows no characteristic alteration in most types of hepatic injury, whereas the cholesterol level drops, owing possibly to decreased cholesterol esterification. Although the vitamin A content is usually reduced, occasionally it is increased. In carbon tetrachloride intoxication, areas of liver damage retain vitamin A in deficiency states longer than normal areas [2643].

ENZYMES. The activity of hydrolyzing enzymes, such as esterase, in damaged hepatic tissue is reduced [919, 2637]. This appears to be the most typical change in hepatocellular injury [1820]. Oxidative enzyme activity is reduced in many types of hepatic injury, whether measured as succinoxidase or tetrazolium dehydrogenase. This reduced activity is also recognized by a decrease in tissue respiration and carbon dioxide production after an initial rise [214]. Alkaline phosphatase activity in hepatic tissue rises in all types of hepatic injury, as demonstrated chemically and histochemically [1869, 3045]. In experimental liver damage, hepatic phosphatase is increased, or at least not reduced [2637]. Hepatic protein is lost [3291]. Alkaline phosphatase activity is increased in necrobiotic hepatic cells, while necrotic cells usually show no phosphatase activity [3445]. The increase in alkaline phosphatase activity in acute and chronic hepatic injury in the face of reduced activity of other enzymes is best explained by the fact that alkaline phosphatase is primarily an extracellular enzyme found chiefly

in the bile canaliculi, bile ducts, and sinusoids (see Hepatic Phosphatase, Chap. 7). The rise of hepatic phosphatase activity and the fall of hepatic esterase activity constitute a sensitive index of hepatic-cell degeneration [2637].

ELECTROLYTES. Changes in mineral concentrations in the liver are probably an expression of an altered permeability of the hepatic cells, permitting the flow of sodium into and of potassium out of the organ [944].

Mechanisms of Hepatocellular Degeneration and Necrosis

CIRCULATORY FACTORS. Many examples of circulatory factors producing hepatocellular degeneration and necrosis are known. Focal necrosis results from occlusion of sinusoids by fibrin or proliferated Kupffer cells [2187], as seen in terminal stages of diseases with poor general circulation [459]. Similarly, central or massive necrosis is said to result from forward failure produced by interference with sinusoidal blood flow, by swollen hepatic cells on the periphery of the lobule, by spasm of the sinusoids [3463], and by interference with hepatic arterial flow. Circulatory forward failure may thus be the main factor in toxic processes. Backward failure results either from cardiac insufficiency or from contraction of the hepatic veins as a result of various intoxications [2175, 3470].

DEFICIENCY FACTORS. The absence of essential substances from the portal blood alters the function and structure of the hepatic cells. Protein and amino acid deficiencies produce hepatocellular degeneration [1319, 2968] (see Nutritional Deficiencies, Chap. 50). Nutritional deficiency is a result not only of reduced intake but also of diversion of the available amino acids for other purposes (see Deficiency of Specific Amino Acids, under Nutritional Hepatic Injury in Animals, Chap. 50). The effect of circulatory factors upon deficiency is illustrated by predominance of necrosis in the left lobe because of streamlines of portal vein flow (see Streamlines of Flow, under Portal Vein, Chap. 18).

TOXIC FACTORS. The mechanism of the effect on the hepatic cells of toxic material brought to the liver either through the portal vein or, less commonly, through the hepatic artery is still conjectural. Some poisons inhibit enzyme activity. Others, such as bromobenzene or ethionine, create a "conditioned deficiency" (see Deficiency of Specific Amino Acids, under Nutritional Hepatic Injury in Animals, Chap. 50).

INFLAMMATION AND INFECTION. The mechanism by which inflammation leads to hepatic necrosis is unknown. This may be another example of focal, zonal, or massive interference of sinusoidal blood flow or invasion of cells by microbial agents which destroy them, with resulting focal necrosis.

HEPATOCELLULAR DEGENERATION AND NECROSIS: FUNCTIONAL ALTERATIONS

Hepatocellular degeneration ranges functionally and clinically from minimal hepatic insufficiency to fatal hepatic failure. Since clinical, laboratory, and morphologic changes do not necessarily parallel each other, a detailed combined grading, although extremely desirable, is as yet not possible. It is more practical to distinguish only three degrees of hepatic-cell degeneration: (1) sub-clinical hepatic insufficiency, in which chemical or morphologic procedures indicate an abnormality without significant clinical signs or symptoms; (2) hepatic insufficiency characterized by both laboratory and clinical evidence of hepatic-cell degeneration; (3) severe hepatic failure in which the signs of hepatic coma, usually with a characteristic encephalopathy, are present and the immediate prognosis is grave.

CHEMICAL CHANGES IN SERUM AND URINE IN HEPATIC-CELL DEGENERATION

Transitions exist between hepatic insufficiency and severe hepatic failure, and differentiation between the two by chemical tests is often not possible.

Serum Changes. The chemical alterations in the serum that occur in hepatocellular injury are the theoretical basis for the so-called "liver-function" tests. The application of these alterations will therefore be discussed in detail in Part III, and the subject is only briefly reviewed here. Elevation of the serum level of a substance in the presence of liver injury suggests that the substance is formed by extrahepatic tissues and excreted or destroyed by the liver. A drop of the serum level of a substance in the presence of liver injury suggests that the substance is formed by the liver (Table 10).

CARBOHYDRATE METABOLISM. Glucose uptake and glycogenesis from the blood by the damaged liver are reduced. Consequently a tendency to hyperglycemia exists following oral or intravenous glucose administration, reflected in a "diabetic" type of glucose-tolerance curve (see Blood Glucose, under Tests Based on Carbohydrate Metabolism, Chap. 35). For the same reason intermediary carbohydrates such as pyruvate and lactate may accumulate in the blood [62]. They probably come from the extrahepatic tissues and are normally taken up by the liver to form glycogen. Since the formation of blood glucose is one of the most essential functions of the liver, fasting hypoglycemia is rare. Other functions are sacrificed to maintain the normal blood-sugar level, and in the absence of glycogen stores the liver uses all available sources for glucose formation, resulting in a reduction of formation of such substances as urea, albumin, and nucleoprotein. Many metabolic alterations in hepatic injury may thus ultimately be caused by the alteration of the carbohydrate metabolism. Formation of substances derived from the metabolic pool may be reduced, because the intermediates in the pool, such as alpha-ketoglutarate, are used for glucose formation. The metabolism of intermediates such as citrate may be impaired, and citrate intoxication with serious depression of serum-calcium levels can occur in cirrhosis as a result of vigorous transfusion therapy with citrated blood [439]. The occurrence of this complication, however, is rare.

FAT METABOLISM. The serum-lipid fractions are reduced in hepatocellular damage, because a great part of these fractions is produced by the liver. The level of the ketone bodies in liver disease is not fully established.

PROTEIN AND NITROGEN METABOLISM. The reduction of serum albumin, alpha globulin, and prothrombin, as well as of other proteins related to blood coagulation, is the result of decreased formation by the damaged hepatic cells. In mild injuries

and in cirrhosis, fibrinogen is increased [1073] while in severe injury or after hepatectomy it is decreased. The protein moiety of mucoproteins is reduced in human hepatic injury, supposedly because of impairment of alpha globulin forma-

Table 10 Practical Diagnostic Significance and Mechanism of Changes in Levels of Various Metabolites in Blood as a Result of Hepatic-cell Degeneration

Substance	Direction of change	Change primarily result of disturbed hepatic-cell function	Practical diagnostic significance	Mechanism of alteration
Carbohydrates:				
Glucose.....	↓	+	Hyperglycemia and glycosuria may be present; hypoglycemia is occasional preterminal event	Liver maintains blood-glucose level
Lactate }.....	↑	+	Limited	Liver removes lactate and pyruvate to form glycogen
Pyruvate }.....	↑	+	Limited	Part of metabolic pool
Ketoglutarate.....	↑	+	Limited	Part of metabolic pool
Citrate.....	↑	+	Limited	Part of metabolic pool
Oxalate.....	↑	+	Limited	Part of metabolic pool
Lipids:				
Total lipids.....	↓	+	Hyperlipemia indicates associated impairment of bile flow	Chiefly due to drop of cholesterol and phospholipids
Phospholipid }.....	↓	+	When elevated, impairment of bile flow; cholesterol may increase in early acute damage	Liver produces large portion of these lipids and excretes them in bile
Cholesterol }.....	↓	+	Sensitive indicator of damage, even in face of impaired bile flow	Liver responsible for portion of esterification
Cholesterol ester.....	↓	+	In severe damage only	Liver produces ketone bodies; variable changes in lesser degrees of damage
Ketone bodies.....	↓	+	Limited	Liver maintains blood level; serum level drops after stores depleted
Vitamin A.....	↓	?	Limited	Liver maintains blood level; serum level drops after stores depleted
Proteins and nitrogen:				
Albumin.....	↓	+	Valuable indicator of more protracted damage	Liver produces serum albumin
Globulin }.....	↓	-	Indicates mesenchymal reaction to parenchymal damage	Produced in part by stimulated Kupfer cells
Gamma globulin }.....	↓	+	Limited	Probably produced by liver
Alpha globulin.....	↓	+	Coagulation factors, absence of which leads to hemorrhagic diathesis; prothrombin deficiency due to damage does not respond to parenteral vitamin K	All produced by liver; fibrinogen is increased in mild injuries and in human cirrhosis
Prothrombin }.....	↓	+	Limited	Produced by liver
Fibrinogen }.....	↓	+	May aid in differentiating tumors from damage	Possibly impaired alpha globulin formation by liver
AC globulin }.....	↓	+	Indicates associated renal impairment	Produced largely by liver especially urea; blood urea nitrogen drops in hepatectomized animals and in complicated severe hepatitis because liver produces urea
Complement.....	↓	+	Limited	Liver deaminizes amino acids
Mucoprotein.....	↓	+	May aid in differentiating tumors from damage	Ammonia transport mechanism in body. Liver splits it into glutamic acid and ammonia
Nonprotein nitrogen }.....	↓	-	Limited	Liver removes ammonia to form urea
Blood urea nitrogen }.....	↓	-	Limited	Excreted in bile and probably also formed in liver in part
Amino nitrogen.....	↑	+	Indicates severe liver damage	Produced by liver
Glutamine.....	↑	?	May be related to hepatic coma	Apparently related to retention of biliary constituents
Ammonia.....	↑	?	Severe damage necessary	Probably produced by liver
Enzymes:				
Phosphatase.....	↑	-	Much elevation means impairment of bile flow; slight elevation common in liver damage	Produced by liver
Esterase.....	↓	+	Sensitive indicator of damage	Apparently related to retention of biliary constituents
Hyaluronidase inhibitor.....	↑	-	Limited; drops in hepatic coma	Probably produced by liver
Xanthine oxidase.....	↑	+	Of value in experimental rats	Probably produced by liver
Minerals:				
Sodium.....	↓	-	Limited	Moves into damaged cells
Potassium.....	↓	-	Limited	Moves out of damaged cells but may be decreased because of dietary restriction
Phosphorus }.....	↓	-	Limited	Decreased probably because of dietary deficiency
Calcium }.....	↓	-	Limited	Inadequate destruction of cells in liver
Protein-bound iodine.....	↑	?	Elevated in acute damage	Unknown
Iron.....	↑	?	Elevated in acute damage	Unknown
Bile salts and pigments:				
Cholates.....	↑	?	Limited	Produced by liver and excreted in bile
Bilirubin.....	↑	?	Widely used to follow progress of liver damage	Excreted into bile, bilirubin in liver damage may be due to decreased removal from blood, faulty excretion through Kupfer and other cells, or associated impairment of flow (regurgitation)

tion. The tendency for reduced blood-urea levels, from faulty formation by the damaged liver, is overshadowed by urea retention resulting from reduced renal function [2277]. This also results in elevation of NPN, amino nitrogen, and creatinine. The uric acid level is especially elevated in hemorrhagic necrosis of the liver following eclampsia and in toxemias of pregnancy in general. In liver disease, the uric acid level is often low. Serum ammonia, which is transformed by the liver to urea, is increased in severe hepatic injury and in cirrhosis, regardless of the condition of the patient. This elevation, however, may actually be caused by an increase in the blood of various amines which are determined as ammonia. The increase in glutamine, the form in which ammonia is transported to the liver, is related to the impaired urea-forming mechanism in the liver [3475]. Elevated levels of the blood and brain glutamine and ammonia may be in part responsible for the signs and symptoms of hepatic coma (see Hepatic Coma, further on in this chapter). Penicillamine is found in the urine of patients with liver disease receiving penicillin, whereas it is not found in persons without liver damage [3475].

ENZYMES. Alteration of the serum-enzyme activities as a result of hepatic damage is a sensitive indication of hepatocellular degeneration, although further work is required for better understanding of the specificity of these changes. Serum cholinesterase activity is reduced in human hepatocellular damage [1212, 3444]. In chronic liver injury produced experimentally, this reduction is not consistently found, and occasionally even an elevation is observed. If the esterase activities are followed at short intervals after the onset of bromobenzene or carbon tetrachloride intoxications, an initial rise above normal is seen, followed by abnormally low values [2637]. This biphasic curve is explained by an initial release of hepatic esterase from the damaged hepatic cells to the serum, followed by a decrease when hepatic formation is depressed. With the exception of the initial stage, serum esterase reduction thus measures hepatic depletion. A similar excessive release is also suggested by the results of xanthine oxidase determinations. This enzyme is found in rats but not in man. A subsequent depletion in chronic injury is not observed, however. Alkaline phosphatase activity behaves differently in that it is slightly increased in parenchymal degeneration, in contrast to the large

increase in cholestasis (see Serum Alkaline Phosphatase, Chap. 7) [1314]. It is supposedly decreased in severe hepatic failure [2640]. In acute experimentally produced hepatic injury the serum activity of alkaline phosphatase rises rapidly [2637]. This rise is maintained or goes even higher in chronic hepatic injury.

The increase of serum hyaluronidase inhibitor in liver disease is possibly caused by retention of biliary constituents [3121], although the reduction in severe hepatic failure may be the result of impairment of specific hepatocellular function.

MINERALS. The disturbed permeability of the damaged cell membranes should lead to increased serum potassium and decreased serum sodium in liver damage. However, nutritional deficiencies, loss of body fluids by vomiting and diarrhea, as well as renal disturbances, usually result in loss of all serum electrolytes, particularly in hypokalemia [64]. The elevation of protein-bound iodine in acute hepatitis has been explained as the result of inhibition of the normal ability of the hepatic cells to destroy thyroxine [1890]. The normal values found in cirrhosis, however, are supposedly the result of regeneration of hepatic cells. So far, the elevation of serum-iron level in liver damage, especially in acute hepatitis [2576] and experimental carbon tetrachloride intoxication in dogs [2739], is not satisfactorily explained.

BILIRUBIN AND BILE SALTS. The elevated levels of serum cholates and bilirubin are probably expressions of both disturbed excretion by the hepatic cells and regurgitation.

Chemical Changes in Urine. The implications of the urinary alterations in hepatic injury (Table 11), such as bilirubinuria (see Urinary Excretion of Bilirubin, Chap. 11), in most instances are obvious and have been discussed. The increases

Table 11 Substances Found in Urine in Increased or Decreased Amounts, or Abnormally Present, as a Result of Hepatic-cell Degeneration

<i>Increased</i>	<i>Decreased</i>	<i>Abnormally present</i>
Urobilinogen Coproporphyrin Estrogens (in some cases)	Chlorides Sodium 17-ketosteroids Androgens Gonadotropins	Bilirubin Amino acids Leucine Tyrosine Cholates Creatine

in urinary urobilinogen and amino acids are an indication of severe hepatic injury and reflect the serum elevation, which is less easily demonstrable [3475]. The increase is primarily caused by increases in methionine, tyrosine, and valine, in addition to leucine and cystine [863]. In rare instances ethanolamine is excreted [773]. The 17-ketosteroid and urinary gonadotropin excretions are reduced, while the estrogen excretion does not show consistent variations [2599] (see *Effect of the Liver on Estrogens*, Chap. 62). Creatinuria has been reported in diseases of the liver. Increased amounts of urinary coproporphyrins I and III are found [2430] (see *Porphyryns*, Chap. 36).

Chemical Changes in Severe Hepatic Failure

The experimental example of hepatic failure is total hepatectomy, based on the classic studies of Mann, Bollman, and Magath [2202], supplemented by the findings in dogs with Eck fistulas (see *Eck Fistula*, under *Portal Vein*, Chap. 18). These are contrasted with findings in human terminal hepatic failure.

Hepatectomized Animals. The following changes characteristically occur after hepatectomy [2202]. The blood-sugar level drops and must be maintained by glucose administration in order to preserve the animal long enough to study the subsequent changes. Glucose tolerance decreases as time goes on. The blood- and urine-urea levels decrease, while the amino acid and uric acid levels rise. The blood levels of all amino acids rise, the greatest increase being that of glutamine, which is normally the most abundant one in plasma [1036]. The glutamine content of the brain increases greatly, while that of the muscle increases only slightly [1033]. The specific dynamic action of alanine and glycine is abolished, and the tissue amino acid concentration does not rise on amino acid administration. The blood-ammonia level increases only slightly, and the ammonia excretion remains normal. No change occurs in either blood or urinary creatinine. Fibrinogen, prothrombin, cothromboplastin, and labile factor levels drop [2205]. Cholesterol remains unchanged, probably because the animals do not live long [2202]. The electrophoretic total protein pattern is not significantly altered [1985] except for a drop in α_1 globulin [1986]. The serum-alkaline phosphatase activity rises, although less than after common duct ligation [1088]. The serum-bilirubin level rises steadily, and bilirubin appears in the urine

shortly after the procedure and continues to increase [2202].

ECK FISTULA. A partial experimental reduction of hepatic function results from the Eck fistula, in which the blood of the portal vein is directed into the inferior vena cava. Animals with such fistulas are unable to synthesize plasma protein, hemoglobin, and bile salts, to store fluid, or to destroy uric acid, although glucose utilization remains intact [1358]. Dye clearance is impaired and alkaline phosphatase activity is also increased.

Severe Human Hepatic Failure. The biochemical changes in human hepatic failure have attracted interest for the recognition of imminent hepatic breakdown by laboratory methods. No characteristic differences have been found, however, which would permit the recognition of the preterminal stage [495, 2387, 3282]. Certain findings, especially when followed serially, indicate a deterioration of hepatic function, but the same findings are noted in patients who survive or are not in coma [3116]. Although the findings listed below are not necessarily specific, they are usually considered indicative of severe hepatic failure. Some of them differ from the alterations in the hepatectomized dog, and many of the alterations are associated with inanition and shock rather than with liver damage.

NITROGENOUS SUBSTANCES. Elevation of the level of amino acids in the blood is associated with increased urinary excretion of all amino acids [3475]. Tyrosine can be demonstrated by the Millon reaction or by specific chemical methods, and amino acid crystals, supposedly those of leucine and tyrosine, are seen by microscopic examination [1996]. In contrast to the condition in hepatectomized animals, the blood-urea level in man is frequently not decreased but actually elevated as a result of associated renal impairment [2277]. The serum nonprotein nitrogen (NPN) is almost always increased, even if the urea level is not elevated, because of the rise of other nonprotein nitrogen-containing substances, particularly the amino acids. The NPN elevation is more pronounced in instances with a fatal outcome. The creatinine level does not rise so high as the levels of blood-urea nitrogen (BUN) and nonprotein nitrogen, implying an increase in tubular resorption rather than a decrease in filtration [2277]. The rise of the NPN is higher than the BUN in some instances, and a high NPN/BUN ratio is considered a sign of catastrophic hepatic failure [2085]. In cirrhosis, the blood-ammonia

level is elevated whether coma is present or not, and little correlation is found between the ammonia level and the degree of consciousness [2963] (see Hepatic Coma, later in this chapter).

LIPIDS AND MINERALS. The drop of the cholesterol ester percentage, with almost complete disappearance of the ester fraction [2195], probably represents the most important biochemical criterion of severe failure. This is followed by a drop in the total cholesterol, phospholipids, and total lipids [3116]. Serum sodium and chloride are both diminished [3106]. Hypopotassemia is common [3696], and radioisotope studies have shown that the total amount of exchangeable potassium is low, and if ascites is present, this is not corrected by oral potassium administration [28].

OTHER CHANGES. A sudden rise in level of the serum bilirubin occurs in some instances of severe failure [3185]. A terminal drop in blood-sugar level is usually obscured by both pancreatic and stress reactions on one hand and therapy on the other; therefore, hypoglycemia is infrequently found in human hepatic disease [3724]. Blood-pyruvate level is usually elevated [62, 495]. The spinal fluid shows no characteristic aberration, even in severe coma, except for increased pyruvate [495] and the occasional appearance of bilirubin.

CLINICAL MANIFESTATIONS OF HEPATIC INSUFFICIENCY

Hepatic insufficiency is clinically reflected in a series of symptoms which culminate in a dramatic fashion in the picture commonly designated as "cholemia." The symptoms characteristic of the milder forms are vague. Anorexia is the most common symptom of hepatocellular degeneration. This is associated with a change in taste, a dislike for tobacco, intolerance for fat, flatulence, and belching. Nausea, vomiting, and diarrhea accompany more severe degrees of hepatic failure. Elevation of temperature is rarely seen in mild hepatic insufficiency without infection but frequently occurs as a terminal event, when it may become very high. Bradycardia, which is usually found in jaundice, may disappear terminally, while cardiac output is increased. The liver may or may not be tender and enlarged. Conspicuous central nervous system manifestations imply a grave prognosis, especially if associated with a hepatic fetor and hemorrhagic tendencies. The condition is described either as cholemia, empha-

sizing the faulty excretion of biliary substances, or as hepatic coma or precoma, emphasizing the neurologic aspects. In the individual case it is often difficult to separate the two features. The term "cholemia" is poorly defined and is often used, as is "hepatargy," to describe the clinical picture seen in hepatic failure. This condition is not always associated with a significant increase of biliary substances in the blood and may occur without jaundice but is then usually accompanied by extensive collaterals, shunting blood from the liver and visible in the abdominal wall.

Hemorrhagic Diathesis

The development of our knowledge of the hemorrhagic diathesis in severe liver disease entailed the detection of more and more factors necessary for the clotting of blood that are either produced by the liver or influenced by hepatic injury. Many of the newly found factors are in the plasma and are part of a system of substances acting synergistically and antagonistically. In order to present the problem fully all aspects of blood coagulation would have to be discussed. Since many reviews and chapters on hematology are devoted to this problem [78, 1039, 2984, 3176], only the main aspects of hepatic involvement are emphasized here. The original scheme of Howell has been extensively modified, and the present knowledge appears well summarized in schemes by Quick [2677] and Stefanini [3176] (Fig. 94). The following substances in blood are altered as a result of hepatocellular degeneration: (1) fibrinogen; (2) prothrombin complex, composed of prothrombin, factor VII, and AC-globulin; (3) platelets; and (4) thromboplastin. Capillary fragility is also altered.

Fibrinogen. Fibrinogen is not significantly reduced in human hepatic disease, except in severe, fatal conditions. In contrast to earlier concepts, therefore, fibrinogenopenia is not an important factor in most instances of hemorrhagic diathesis in man.

Prothrombin Complex. Since the discovery of vitamin K, deficiency of prothrombin has been associated with the hemorrhagic diathesis in hepatic disorders. Recently a deficiency in factor VII that seems to depend on vitamin K more than on prothrombin has been implicated in hepatic disorders [1051, 2506]. Also a deficiency of AC-globulin occurs in liver diseases. Only when the proper differentiating techniques are used are defects in the individual substances demonstra-

ble. Therefore many of the statements concerning prothrombin may refer to one of the other factors.

The plasma protein, prothrombin, is formed solely by the liver under the influence of vitamin K. Vitamin K occurs commonly in the diet and is formed in the intestinal tract by bacteria, but it is sufficiently absorbed only in the presence of bile salts [403, 404, 2610]. The absence of these salts in obstructive jaundice and in some parenchymal liver diseases explains the hypoprothrombinemia often observed [451, 2678, 3488]. Enough vitamin K is absorbed, however, in the absence of bile salts to prevent the prothrombin concentration from dropping to zero [2679]. This has been said to account for the slow fall in prothrombin in congenital atresia of the bile ducts after several months of life [2679].

The defect of vitamin K absorption is corrected either by administration of bile salts orally, thus permitting the absorption of vitamin K, or by parenteral administration of synthetic substances with vitamin K activity which are water-soluble [78, 2082, 3731]. These soluble substances contain the active naphthoquinone group without the side chain and completely correct a hypoprothrombinemia based solely on insufficient intake or absorption of vitamin K, although not so efficiently as the natural vitamin [2679]. Hypoprothrombinemia with faulty response to vitamin K therapy indicates disturbed hepatic function. This

response has been utilized as a hepatic-function test [2640] (see Response of Prothrombin Time to Vitamin K Administration, Chap. 34). The time interval required for the response to vitamin K is variable. The correlation with hepatic function is not good [2082].

Most methods in clinical use for the determination of prothrombin, such as Quick's one-stage method, actually measure a defect of the total prothrombin complex rather than prothrombin specifically (see One-stage Method, under Prothrombin Complex Activity, Chap. 34). If the simple, less specific one-stage method is replaced by the more specific two-stage method, the correlation with hepatic injury is better [2204].

AC-globulin is demonstrable by the two-stage method for prothrombin. It is reduced by hepatocellular degeneration of short duration, such as acute carbon tetrachloride intoxication [3283]. Its concentration parallels that of plasma prothrombin but usually returns to normal more rapidly than that of prothrombin. Factor VII is the most sensitive to liver damage, followed by prothrombin, while AC-globulin is the least sensitive.

Excessive amounts of prothrombin supposedly occur in some instances of hepatic disorders [2709]. High doses of vitamin K are reported to decrease prothrombin rather than increase it in the presence of hepatic injury [3390, 3391].

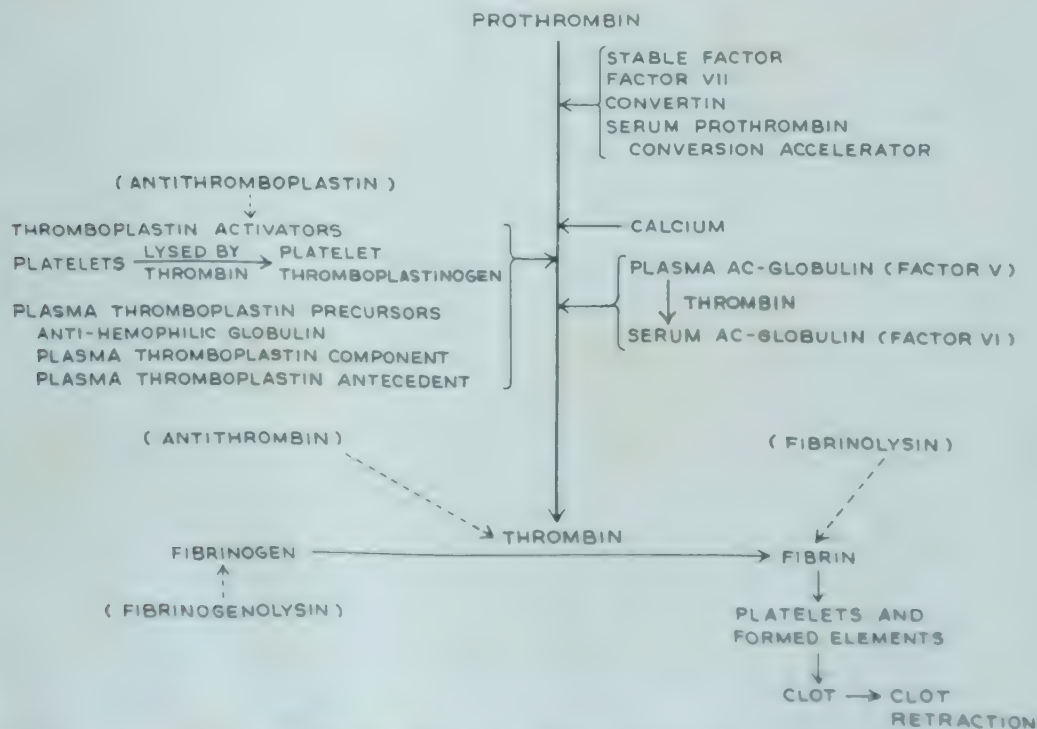


FIG. 94 Theory of blood coagulation.

Idiopathic hypoprothrombinemia may explain some unexpected abnormal results [675, 1176, 2677]. The anticoagulant action of Dicumarol has been linked to liver damage, and preexisting liver damage greatly sensitizes animals to Dicumarol [2754].

THROMBOPLASTIN. Thromboplastin, which is a lipoprotein [549] probably related to coagulase-globulin, is reduced in liver disease [2282].

PLATELETS. Low thrombocyte counts occur in hepatic disorders [2350, 3670] and have been held responsible for part of the hemorrhagic diathesis which is not affected by administration of vitamin K or plasma. Hypersplenism associated with portal hypertension (see Hypersplenism, under Portal Hypertension, Chap. 29) produces thrombocytopenia; this possibly is the explanation for thrombocytopenia in liver disease.

CAPILLARY FRAGILITY. The etiology of capillary hemorrhage is still not fully understood. It may be caused by damage of the endothelial cells, the intercellular cement substance [539, 2276], or the external basement membrane of connective tissue. No evidence exists for generalized capillary endothelial damage in liver disease; nor can involvement of the connective or elastic tissue around the capillary be shown [3591].

Sequelae. Hemorrhagic diathesis requires energetic treatment, particularly if surgical intervention or liver biopsy is contemplated. Rarely ascitic fluid is hemorrhagic in cirrhosis with bleeding tendencies [2199]. Bleeding increases jaundice by hemolysis and leads to azotemia, because the liver is unable to cope with the increased amount of breakdown products. Pulmonary emboli allegedly occur less frequently in hepatic disease, particularly in cirrhosis, because of the reduced coagulability of the blood [3160].

Fetor Hepaticus

One of the most reliable and conspicuous signs of hepatic failure is the fetor hepaticus, the smell being similar but not identical to that of alpha-methylpiperidine [450]. The presence or absence of the fetor is not related to the degree of jaundice or of impairment of hepatic function. The substance responsible has recently been identified as methylmercaptan [536]. It is supposedly derived from excess methionine; therefore the fetor may indicate an inability of the liver to metabolize methionine. It is found in the urine in normal individuals and in patients with liver disease and has been concentrated [3502]. It is reduced after a

bowel movement or during diarrhea, suggesting an intestinal origin, but sterilization of the stool with antibiotics does not affect it. Since the disappearance of the fetor may precede recession of coma, the odorous substances may be responsible for some of the signs of hepatic coma. Fetor hepaticus is found twice as frequently in patients with hepatocellular jaundice as in patients with extrahepatic biliary obstruction [450].

Ascites

In the formation of ascites in liver disease, especially in cirrhosis, hepatocellular degeneration plays an important role. It interferes with inactivation of pituitary or adrenal hormones, particularly aldosterone, producing sodium and/or water retention and the formation of serum albumin. Portal hypertension is another important factor in ascites formation in liver disease; ascites occurs mainly in liver diseases associated with portal hypertension. Ascites formation is discussed, therefore, as a sequela of portal hypertension (see Ascites, Chap. 29).

Pulmonary Edema

Pulmonary edema, not necessarily resulting from failure of the left side of the heart, occurs commonly as an expression of disturbed water and salt metabolism as well as of altered capillary permeability in liver disease. As in uremia, a multitude of factors seems to play a role, including acidosis and dehydration, even in the presence of ascites.

Hepatic Coma

The central nervous system manifestations associated with severe liver disease, although not necessarily with jaundice, are designated "hepatic precoma" or "coma" depending on their severity. They imply a serious prognosis.

Clinical Manifestations. The clinical picture varies between somnolence and lethargy, gradually increasing to a stuporous coma on one hand and motor hyperactivity, ranging from noisy confusion to psychosis or convulsions, on the other hand [448, 1270, 2387, 3116, 3475]. The manifestations vary greatly from patient to patient and from day to day. The symptoms are reversible and are sometimes reversed within a very short time. The central nervous manifestations and the general behavior of the patient depend largely on the stage of the disease in which hepatic coma develops. In acute diseases the behavior changes rapidly; whereas in chronic diseases, such as cir-

rhosis or obstruction, inconspicuous initial changes occur which may be overlooked for several days. Allegedly in cirrhosis or hepatitis, the unconsciousness is not necessarily absolute and the patient may even be able to swallow, while in the primary obstructive forms the patient is restless and can not be aroused [1996].

Impending coma is characterized by a triad of mental confusion, "flapping" tremor, and electroencephalographic changes. Lack of alertness, somnolence, and increased duration of sleep are followed by a noisy state of confusion, which persists for a period of time as a frank psychosis.

Flapping tremor, a flexion-extension movement of the hand and wrist best elicited in the outstretched hand, is a grave prognostic sign [14, 2961].

Characteristic electroencephalographic changes with delta wave activity have been described in impending coma [448, 1042, 2961]. They are more common in children and more severe if associated with other diseases [2387]. The comatose state is usually characterized by the lack of any voluntary movement and failure to respond even to strong stimuli. The motor changes are not related to the somnolence, and hyperactivity of reflexes and spasticity do not necessarily parallel alterations of behavior [3475]. The limbs of the patient may be flexed and may have a peculiar clasp-knife rigidity [1497]. Pyramidal signs are the most common neurologic abnormality. Spasticity and muscular spasm are found, probably associated with changes in the basal ganglions. "Cholemic crying" is noted in children. Convulsions are infrequent; when they occur, they may be caused by hypoglycemia [1497, 3116]. The duration of both the precomatose and comatose states varies from several hours to several days. This picture may be complicated by other diseases, especially infections, and by medications, particularly barbiturates [2387].

Anatomic Changes. Anatomically, the central nervous system manifestations are not specific. Edema is found, along with degeneration of ganglion cells. Focal and diffuse proliferation of the glial cells in the gray and white matter is noted. Large glial nuclei practically free of cytoplasm are described [2570]. Focal hemorrhages in the brain are the exception rather than the rule [3235].

Mechanism of Hepatic Coma. Hepatocellular degeneration is an obvious cause of cholemia and coma, although which function is failing is un-

known. Bypass of the hepatic parenchyma by the intrahepatic portohepatic venous anastomoses and by the extrahepatic portocaval anastomoses, allowing nitrogenous substances from the intestine to reach the general circulation, is a contributing factor. It may appear arbitrary to separate cholemia as an expression of generalized intoxication from hepatic coma as an expression of central nervous system intoxication, and many clinicians identify cholemia as precoma. In hepatectomized dogs, cholemia but not coma develops.

Hepatic coma occurs in acute hepatitis, in cirrhosis, and in the terminal stage of obstructive jaundice. Experimentally, it has been produced in dogs by greatly reducing the hepatic blood flow from the hepatic artery and portal vein in three stages [2710]. The liver shows extensive central necrosis, and the dogs are in coma for several days. They show loss of appetite, vomiting, restlessness, stupor, somnolence, and finally coma with muscular twitching. With development of collateral circulation the hepatic changes regress and the dogs recover from the coma. The rapid reversibility, as well as the nonspecific morphologic central nervous system manifestations, strongly suggests a biochemical defect as the cause of the condition. Several hypotheses for the mechanism of hepatic coma have been presented [448].

1. Breakdown products of liver tissue have been suggested as the cause of acute hepatic failure with hyperpyrexia, coma, and renal insufficiency. This suggestion is supported by the observation that the presence of liver tissue free in the peritoneal cavity is fatal, presumably owing to toxic amino acids. Thus far the toxic substances derived from hepatic autolysis remain unknown.

2. Hypoglycemia, which occurs in the hepatectomized dog, is found in some forms of human hepatic failure and has been considered a cause of hepatic coma. Hepatic coma develops, however, with normal or even increased blood-sugar levels. Nevertheless some of the convulsions in coma may be related to hypoglycemia [1497, 3475].

3. A drop in the level of plasma cholinesterase with a rise in acetylcholine is listed as a cause of hepatic coma [1893]. Raising the cholinesterase level does not, however, materially affect hepatic coma [3475].

4. A disturbance of the intermediary metabolism of carbohydrates may be responsible for hepatic coma, particularly in view of the associated alteration of the metabolism of electrolytes and members of the vitamin B complex [3119].

The elevation of the pyruvate and lactate levels in the blood and spinal fluid is probably the result of inability of the liver to form dicarboxylic acids necessary in cellular aerobic metabolism and in the Krebs cycle [62, 495]. Serum potassium and magnesium levels are lowered [104, 448] although the importance of these substances in the genesis of coma is not clear. Thiamine phosphorylation is impaired [3610]. Nevertheless attempts to use intermediates such as succinate in therapy have been in vain [463].

5. A specific supporting influence upon brain metabolism may be exerted by substances formed by the liver. Hepatic failure may reduce the supply of such substances. Perfusion of the brain with artificial blood mixtures maintains the uptake and oxidation of the carbohydrates for prolonged periods only if the liver is included in the perfusion system, possibly because of the release of a glucosamine-like substance [1141].

6. The presence of excess amino acids, especially methionine, in the blood and urine has been considered a cause of hepatic coma [536, 3475], although this excess is less commonly found in cirrhosis than in hepatitis. The neurologic symptoms have been associated with either a breakdown of the liver mechanism for regulating amino acids or the liberation of amino acids from the autolyzing liver, which in turn are toxic for the brain. The spinal fluid proteins are increased during coma [65].

7. Failure of detoxification increases the concentration of substances in the blood which are normally removed by the liver. The nature of these substances is not established, although many, such as ammonia and the aromatic amines, indole, and skatole, have been accused.

The injury from intestinal substances formed or absorbed excessively, which are either bacterial in origin or protein-breakdown products, has been called autointoxication, or intestinal toxemia. In recent years the theory of autointoxication has found new support. In the presence of liver diseases, intestinal bacteria are found in the liver and even in the blood stream [3576]. Further support was given to the theory by recent experiences with antibiotics [1327, 3016]. However, the lack of response to insoluble sulfonamides and the lack of beneficial effects of antibiotics in liver damage owing to causes other than dietary deficiency have cast doubt on this theory [2744].

Renal failure associated with hepatic failure may lead to a uremic component exaggerated by

faulty detoxification of some of the retained compounds.

Toxic substances normally formed and excessively absorbed, or abnormally formed, may bypass the liver through collaterals. This may occur in cirrhosis through extrahepatic collaterals or through the portohepatic venous anastomoses in the liver (see Vascular Anastomoses, under Processes Common to All Types of Cirrhosis, Chap. 28), even in the absence of jaundice. Surgical portocaval shunts usually do not produce central nervous system manifestations [3475]. In cirrhosis in which abnormal communications between the portal and the systemic circulations develop, central nervous system manifestations including clonus, hyperreflexia, personality changes, and even psychoses may occur and have been called "portosystemic encephalopathy" [3044]. These conditions have improved, sometimes dramatically, on restriction of dietary protein. Brain changes after meat feeding in dogs with Eck fistulas are also listed in support of this theory. Furthermore, stupor has been produced in a patient with a surgical Eck fistula by giving substances which ultimately are converted to ammonia [2113].

8. Ammonia or ammonialike substances appear to be important in hepatic coma. Ammonia coming from the intestine is transformed by the liver to urea, glutamine, and asparagine. The glutamic acid-glutamine balance, related to the ammonia-transport mechanism, is disturbed in hepatic coma [3538], and glutamine and ammonia are increased in the blood in coma [2962, 3353]. Levels of ammonia or ammonialike substances in the blood are elevated in cirrhosis, regardless of the clinical condition of the patient [3086]. This has been thought to indicate the extent of portocaval communications to a greater degree than the amount of liver damage [3587]. Uptake of ammonia by the brain is increased, as indicated by differences in arterial and jugular vein ammonia levels [256]. This possibly causes the removal of alpha-ketoglutarate from the Krebs cycle for glutamine formation, particularly in the brain [256]. Administration of nitrogenous substances to patients with severe liver disease may precipitate coma [2588]. Administration of glutamic acid in hepatic coma in an attempt to bind more ammonia has been tried [3086, 3475], but no effect on either blood-ammonia levels [3086] or the clinical course [463] was seen except in instances where symptoms were produced by ammonia administration [2114].

Ammoniacal Encephalopathy. The excess of ammonia or ammonialike substances reaching the brain as a result of reduced hepatic function or of the bypass of blood around the liver parenchyma appears to produce well-defined neurologic changes. This ammoniacal encephalopathy apparently is the cause of the characteristic triad of hepatic precoma—the somnolence, flapping tremor, and electroencephalographic changes. The disturbance of the ammonia metabolism seems to be effectively controlled by administration of glutamic acid, which binds ammonia and is transformed to glutamine [2114]. In the patient with severe hepatic disease, the hepatic coma may be caused not only by ammoniacal encephalopathy but also by other metabolic disturbances resulting from the cholemia. Therefore, under these circumstances, glutamic acid therapy or reduction of the protein intake to reduce the ammonia in the blood is not effective.

Pure ammoniacal encephalopathy may occur in a person with only a moderately damaged liver but with an extensive bypass of blood around the

liver, as a result of intrahepatic arteriovenous anastomoses, extrahepatic collaterals, surgical shunts, or anomalies. In these instances hepatic precoma may be induced or greatly aggravated by ammonia administration or by a high-protein diet. This form is favorably influenced by glutamic acid administration or protein restriction.

Precipitating Factors. Hepatic coma is usually precipitated by a rapid disintegration of liver tissue [448, 1694]. In acute liver disease, coma is caused by the disease itself, associated malnutrition, or the injudicious use of sedatives. In chronic liver disease, hemorrhage from esophageal varices is an important factor in causing coma. This is chiefly the result of shock and anoxia. Excess nitrogen absorption from the blood in the gastrointestinal tract may be a contributing factor. Hepatic coma may also be precipitated by fatigue with increased metabolic needs, infection, toxic agents, trauma, surgery, or excess nitrogen in the diet. Barbiturates and opiates may precipitate coma [2387]. Excess fluid administration leads to hemodilution, which may also be a precipitating factor.

The mechanisms of biliary obstruction were described in the chapter on jaundice. The consequences are discussed here. These result either from abnormal retention of bile within the intrahepatic biliary passages or from abnormal amounts of biliary substances in the blood stream. Abnormal retention of bile in the bile passages produces mechanical dilatation and stagnation, reflected morphologically in dilated bile canaliculi and ductules. This abnormal retention may also alter the intrahepatic blood circulation or hepatocellular metabolism. Increased amounts of biliary substances in the blood, because of regurgitation or retention, may lead to toxic injury of the hepatic cells. In principle, extrahepatic biliary obstruction produces the same functional and structural sequelae as intrahepatic cholestasis, except for differences in degree. The morphologic consequences are more severe than the functional changes in extrahepatic cholestasis, suggesting that the alteration of metabolism is the same in extrahepatic obstruction and intrahepatic cholestasis, whereas the mechanical effect is much greater in extrahepatic obstruction. The laboratory differentiation between extrahepatic mechanical obstruction and intrahepatic cholestasis is thus usually made by morphologic appearance rather than by laboratory findings.

MORPHOLOGIC APPEARANCE

Certain morphologic features are present in both intrahepatic and extrahepatic cholestasis. They include morphologic signs of bile stasis, enlargement of the liver without significant alteration of the lobular pattern, and inflammation with enlargement of the portal tracts, at least in later stages.

Grossly, the color of the liver varies from brown-green to deep green, depending on the degree and duration of the cholestasis. On the cut surface, the architecture is preserved or even exaggerated, in that the central zones are dark green in contrast to a lighter periphery with moderately enlarged portal tracts. In larger tracts, dilatation of the bile ducts is apparent in extrahepatic cholestasis. The histologic changes of intrahepatic and extrahepatic cholestasis are listed in order as they appear.

Features Common to All Forms

Bile Stasis and Extravasation. The morphologic expression of intralobular cholestasis is the presence of bile-pigmented granules or diffuse bile imbibition in the cytoplasm of the Kupffer cells and hepatic cells, and the dilatation of the bile canaliculi, which contain bile plugs. Eventually bridges of cholestatic tissue connect the central canals. These changes are more prominent in the center of the lobule. In the presence of severe periportal inflammation, bile stasis is conspicuous on the lobular periphery, possibly produced by kinking of the ductules around the limiting plate. The intralobular and perilobular ductules contain larger bile plugs, or microcalculi, and are often dilated, especially proximal to the plug. Rupture of bile canaliculi adjacent to cells with feathery degeneration, or of the ductules, results in bile pigment free in the tissue, or "bile lakes," around which a few scavenger cells, often segmented leukocytes, accumulate (Fig. 96, lower left) (see Hepatic-cell Necrosis, later in this section).

Hepatic-cell Degeneration. Biliary obstruction uncomplicated by infection has been said not to lead to hepatic-cell degeneration [1497]. In contrast, others have noted evidence of hepatocellular

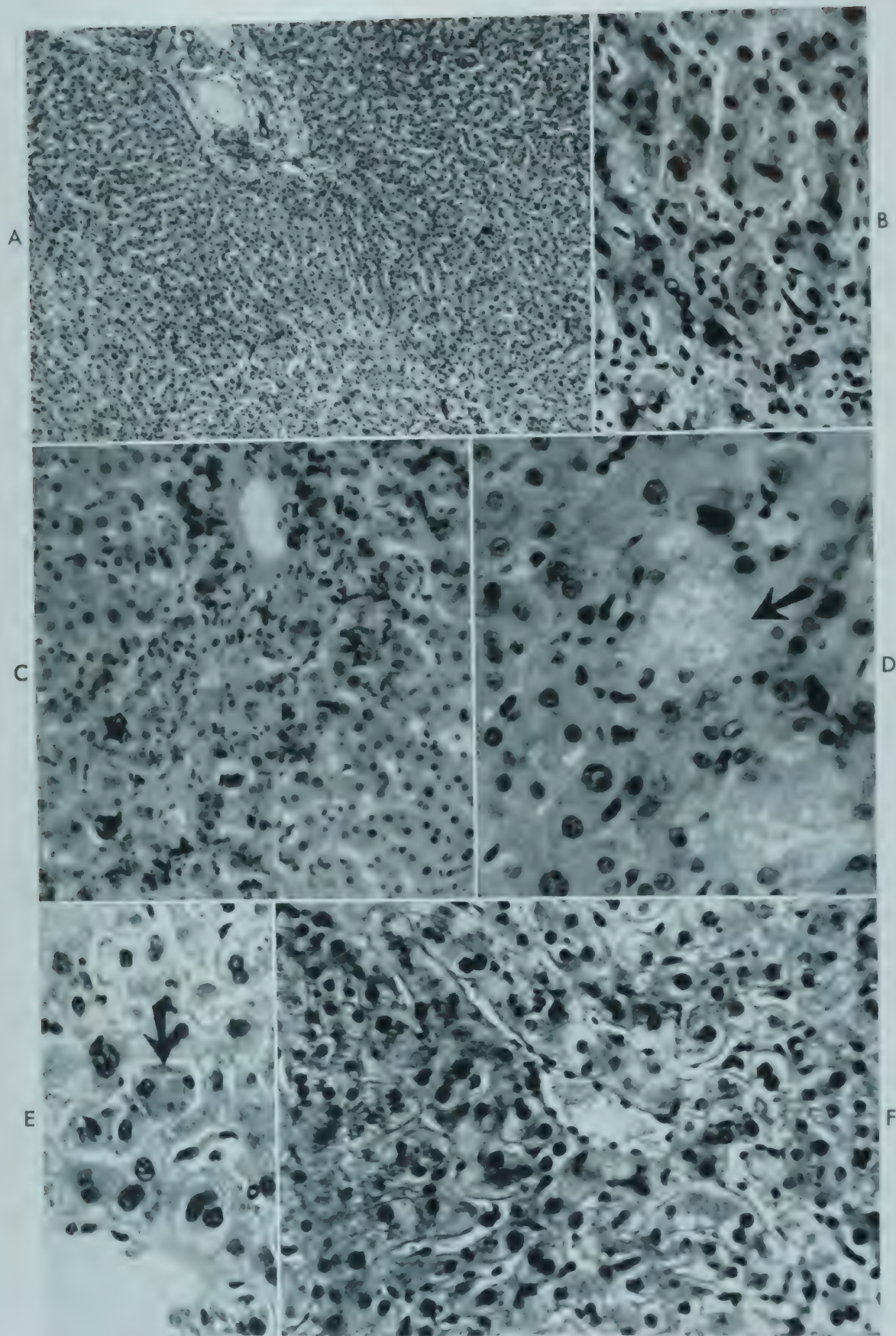


FIG. 95 Biopsy specimens showing early cholestasis caused by extrahepatic biliary obstruction. H&E. A. Complete obstruction, owing to carcinoma of pancreas. Jaundice of 1 week's duration. The portal tract is not altered. In the central zone, bile pigment is deposited and the hepatic-cell plates are thin and irregularly arranged and exhibit some evidence of regeneration ($\times 50$). B. Close-up of central zone. The hepatic-cell plates are thin, and the cells contain pigment. Some binucleated cells are present ($\times 230$). C. complete obstruction of 2 weeks' duration. Much bile pigment is deposited in the central

injury relatively early, especially in liver biopsy specimens [883, 2632, 2802, 3549]. Changes appear as early as 7 days after the onset of complete obstruction. The hepatic-cell plates are reduced in width, especially in the central zone (Fig. 95A, B). This reduction is associated with reduction of glycogen [883], increase of basophilia, increased density of the cytoplasm, and increased perinuclear granularity with the appearance of bile-stained granules. Some cells and groups of cells appear atrophic [1160]. Regeneration is initially subdued but may become intense after several weeks of jaundice (Fig. 95F). Acidophilic degeneration of the cytoplasm develops, and isolated balloon cells with rarefied bile-pigmented cytoplasm appear, chiefly in the central zone (Fig. 95C). The cytoplasm of a few cells around thick bile plugs is poorly stained, and a reticular brown-pigmented film appears (pale or feathery cells) [3549] (Figs. 88F, 95D). In later stages, centrolobular regeneration starts, despite the cholestasis (Fig. 95F). This regeneration can also be seen experimentally after partial hepatectomy [3541]. After complete obstruction for more than 2 weeks, the hepatic-cell plates become irregularly arranged in the center, and the cell shape varies (Fig. 95F). The Kupffer cells are mobilized [3541] and laden with bile pigment. The cytoplasm of the large bile-laden Kupffer cells is not basophilic or pyroninophilic, in contrast to all other types of enlarged Kupffer cells [3287] (Fig. 95E). Whether the bile pigment only masks the basophilia, or whether the pentose nucleic acids are actually absent, as suggested by functional findings, is not known (see Functional Consequences, later in this chapter). A dilatation of the perisinusoidal spaces resembling toxic edema, particularly in the central zone, is observed when hepatic-cell damage is present. After relief of obstruction the pigmentation of the hepatic cells disappears and the hepatic-cell plates return to normal. Bile casts and the pigmentation of the Kupffer cells persist for a considerable length of time, however; occasionally isolated scavenger cells with pigment are noted (Fig. 96, upper left). The degenerative changes of the hepatic cells quickly disappear [467, 1998].

Hepatic-cell Necrosis. Necrosis is found in three forms:

1. Necrosis of isolated hepatic cells. First, nuclear staining disappears, and subsequently the entire cell, while segmented leukocytes accumulate. This necrosis occurs mainly in the central zone, where the bile imbibition is most severe (Fig. 96, upper right). After obstruction of several weeks' duration, these focal necroses coalesce to central necrosis.

2. Necrosis following "feathery degeneration." In either scattered or aggregated cells with feathery degeneration, the nuclear staining disappears, and the cytoplasm of the swollen cells contains a bile-imbibed protein network (reticular biliary necrosis), for instance, as seen in bile infarcts (see Intrahepatic Biliary Obstruction, Chap. 21) (Figs. 98E, 99C). As isolated cells with feathery necrosis disappear and bile canaliculi rupture, the adjacent bile plugs are left outside the biliary passage, and the term "bile lake" is applied (see Bile Stasis and Extravasation, above) (Fig. 96, lower left). The cells surrounding the small necrotic area are not significantly altered.

3. Central necrosis without associated focal necrosis. This occurs chiefly in specimens obtained at necropsy and is probably a terminal event owing to variations in hepatic circulation during the agonal period.

Several factors are responsible for the degenerative and necrotizing changes. A topical chemical effect of one of the constituents of the static bile is suggested by the picture of feathery degeneration near bile plugs and of the subsequent reticular biliary necrosis. Since necrosis also occurs in nonjaundiced parabiotic rats with one common duct ligated [2340], this factor alone is not responsible. Local pressure of bile in bile canaliculi upon hepatic cells best explains the atrophy of the hepatic cells and focal necrosis. Reticular biliary necrosis, however, is not explained. Pressure exerted by proliferating bile ducts and ductules upon hepatic cells is seen in parabiotic rats surviving for long periods after ligation [3697]. The distended and proliferated bile ducts may embarrass the blood circulation [1998]. Such stasis

zone in the form of barely visible granules in the hepatic cells, larger granules in the Kupffer cells, ramified bile plugs, and extracellular masses. Some of the hepatic cells show rarefaction of the cytoplasm ("feathery degeneration") ($\times 170$). D. Close-up showing swollen hepatic cells with rarefied cytoplasm ($\times 315$). E. Bile pigment in Kupffer cells ($\times 220$). F. Degeneration and ballooning of hepatic cells, as well as spotty regeneration in obstruction of 3 weeks' duration ($\times 240$).

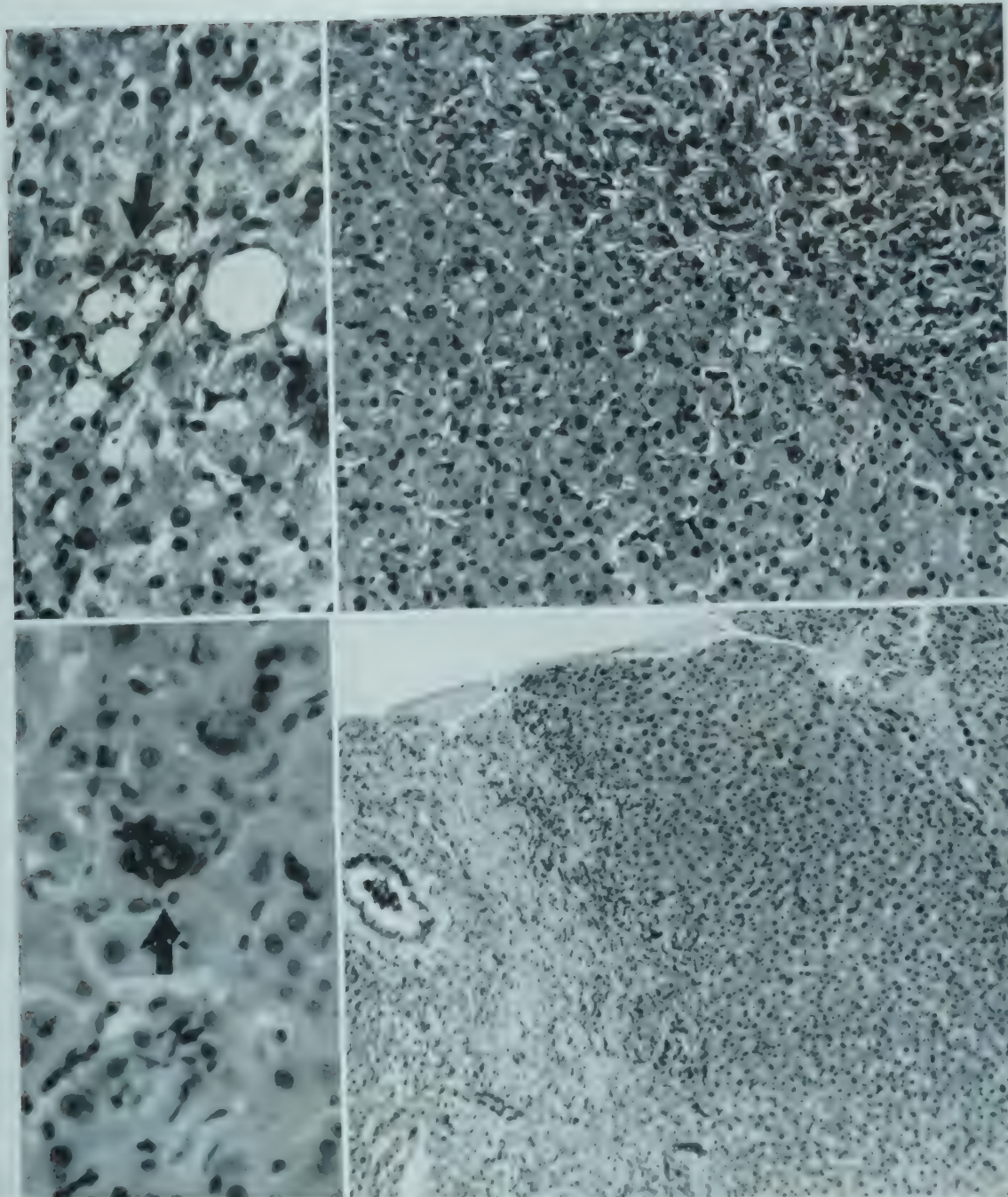


FIG. 96 Biopsy specimens of extrahepatic biliary obstruction. H&E. *Upper left.* Two weeks after relief of obstruction caused by choledocholithiasis. The hepatic cells and Kupffer cells in the center contain small remnants of bile pigment. Aggregates of bile-pigment granules are surrounded by scavenger cells, as well as by vacuoles, some of which contain fat ($\times 230$). *Upper right.* Centrilobular necrosis with ballooning, irregular regeneration, and disappearance of hepatic cells 1 week after relief of obstruction ($\times 110$). *Lower left.* Small intralobular accumulation of bile pigment outside of cells (bile lake) in complete obstruction of 3 weeks' duration caused by carcinoma of the papilla of Vater ($\times 380$). *Lower right.* Scanty inflammatory infiltration of portal tracts, also showing proliferation of the smallest bile ducts, in obstruction of 3 weeks' duration caused by carcinoma of the pancreas ($\times 65$).

in blood vessels has been visualized in corrosion preparations [674].

Hepatocellular necrosis associated with cholestasis occurs predominantly in the extrahepatic form, but beginning stages are seen in the intrahepatic form.

Proliferation of Ductules and Ducts. After prolonged biliary obstruction, usually of several weeks' duration, many more intralobular and portalobular ductules than normally present are visible. In addition, the smallest intralobular bile ducts are proliferated, while the larger bile ducts do not

participate. Injection of the bile duct system shows elongation and increased tortuosity as well as sprouting of the smallest ducts which is much more severe in the extrahepatic form. With prolonged obstruction, the increase in bile ductules becomes very prominent.

In rats with ligated hepatic ducts, bile duct proliferation occurs rather early [883, 1824]. It appears to be caused mainly by mechanical dilatation, since it is not seen in parabiotic partners of rats with ligated common ducts [2340]. In long-standing obstruction, the proliferating bile ducts almost crowd out the hepatic cells [883, 3697]. Ductular proliferation may also be caused by other processes, such as regeneration following necrosis (see *Regeneration after Necrosis of Groups of Nodules*, Chap. 13).

Inflammation. Early intrahepatic or extrahepatic cholestasis is not associated with inflammatory changes in the portal tracts or with edema [1934]. However, after about 10 days of complete extrahepatic biliary obstruction or several weeks of intrahepatic cholestasis or incomplete extrahepatic biliary obstruction, even without evidence of infections, portal inflammation develops (Fig. 96, lower right). This is characterized by the presence of lymphocytes, with a sprinkling of polymorphonuclear leukocytes, around the ductules and an accumulation of round cells, including histiocytes, in the portal tracts, which become enlarged. This inflammation may be complicated by bacterial infection [1497, 2153, 3510]. In very protracted, usually incomplete, cholestasis, the inflammation leads to fibrosis around intralobular and perilobular ductules, which in turn produces kinking and mechanical obstruction of the ductules. This intrahepatic mechanism of biliary obstruction is more common in intrahepatic cholestasis but may occur in late extrahepatic obstruction.

Intrahepatic Cholestasis

The morphology of intrahepatic cholestasis, or cholangiolitis, is not well established, because its pathogenesis is not clear. The concept has been proposed that disturbed permeability of the ductules is the primary lesion, which is followed by obstruction of the ductules by inspissated biliary material, while periductular, or pericholangiolitic, inflammation is secondary to the escape of biliary substances (see *Intrahepatic Biliary Obstruction—Intrahepatic Cholestasis*, Chap. 21).

Early Cholestasis. In earlier stages of intrahepatic cholestasis, bile pigment is deposited in he-

patic cells, Kupffer cells, bile canaliculi, and sometimes in the ductules. This is associated with hepatocellular changes, including feathery degeneration and occasionally bile casts or intralobular bile lakes (Fig. 97A, C). In general, the degenerative changes are less severe and bile lakes are less common than in extrahepatic cholestasis. This is not, however, a reliable differential diagnostic point. In this stage portal inflammation may be missing or subdued. Sometimes the ductules show severe changes and contain inspissated bile in the form of microcalculi, especially in instances of combined intrahepatic cholestasis and hepatic-cell degeneration (Fig. 98B and C). The ductular epithelium appears flattened, atrophic, or even missing in parts of the circumference; in other parts, proliferative changes of the epithelium are noted. Sometimes bile plugs seem to extend to the basement membrane (Fig. 98D). These bizarre pictures are rarely seen in pure intrahepatic cholestasis. When bile plugs in the ductules are very large, small foci of necrobiotic hepatic cells with reticulated bile-pigmented cytoplasm and poor nuclear staining develop within the lobular parenchyma. These foci resemble larger periportal bile infarcts, characteristic of extrahepatic biliary obstruction caused by the dilated intralobular bile ducts interfering with circulation (Fig. 98E) (see *Bile Duct Dilatation*, later in this chapter). Since the intralobular ductules are adjacent to intralobular arterioles, dilated ductules may interfere with the arteriolar blood flow. These small infarcts are seen only in very severe intrahepatic cholestasis and have been observed mainly where the cholestasis complicates severe hepatocellular degeneration, as in viral hepatitis.

Subacute Cholestasis. With protracted intrahepatic cholestasis, the intralobular and periportal ductules appear increased in number and are described as proliferated. This is not necessarily from sprouting from preexisting ductules, but is more likely from transformation of hepatic cells into ductules (see *Origin of "Proliferated Ductules,"* under *Regeneration of Bile Ductules and Ducts*, Chap. 16) (Fig. 97B, E). The absence of mitosis supports such an assumption. The increase in ductules is usually associated with extensive inflammatory exudate consisting of round cells with a variable sprinkling of segmented neutrophilic and eosinophilic leukocytes (Fig. 98A). In hypersensitivity reactions produced by drugs such as chlorpromazine, methyltestosterone, or arsenicals, eosinophils may be more conspicuous, although

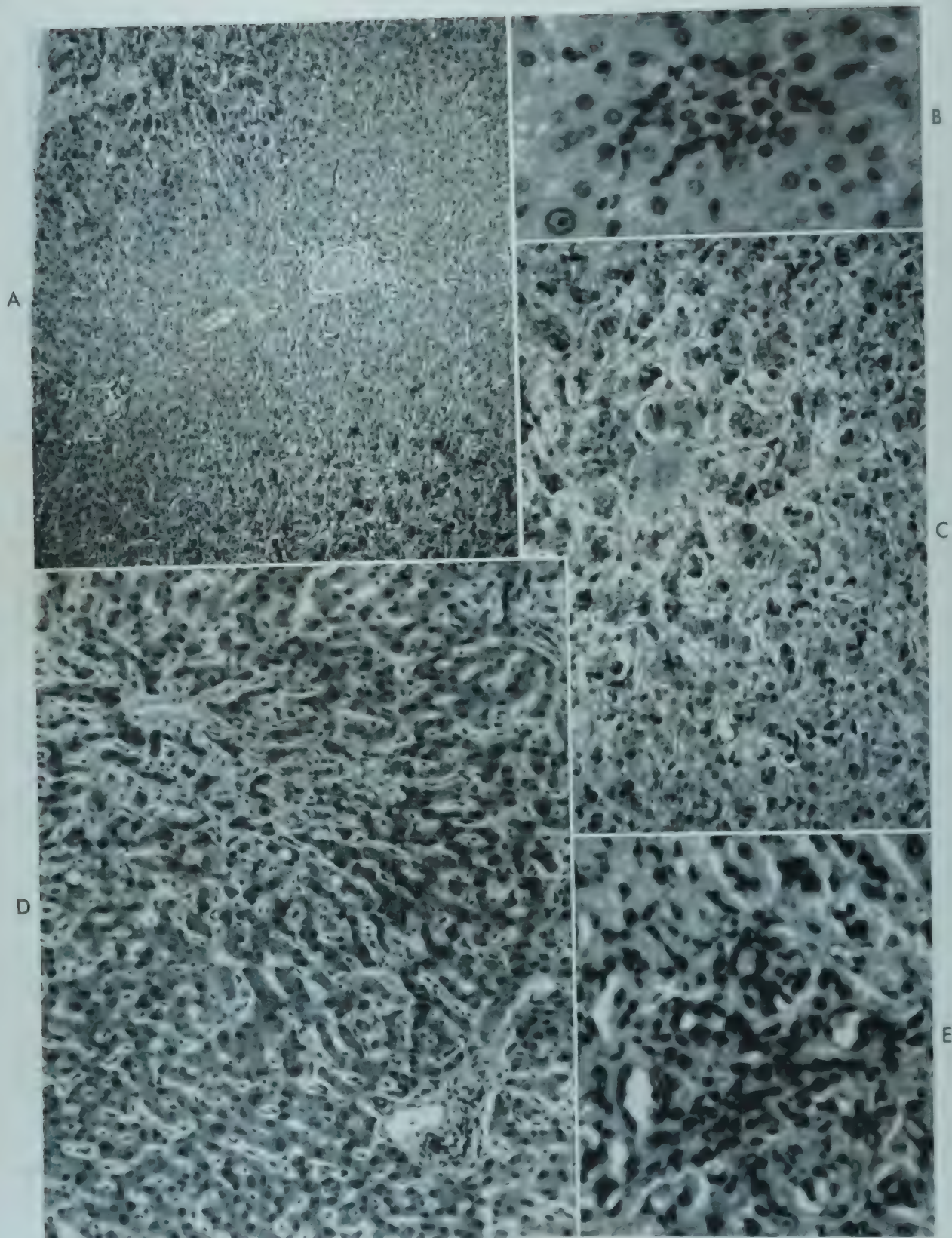


FIG. 97 Intrahepatic cholestasis (cholangiolitis). A, B, C, and E are biopsy specimens. H&E. A. Centrolobular bile stasis indicated by the darker hue. Portal tract in center of picture is devoid of inflammatory exudate ($\times 55$). B. Close-up of intralobular bile ductule surrounded by a few exudate cells. Hepatic cells appear normal. C. Severe centrolobular bile stasis with some ballooning of hepatic cells ($\times 115$). D. Autopsy specimen obtained several days after exploration for intrahepatic cholestasis of 2 weeks' duration. Except for terminal central necrosis, the arrangement of the hepatic cells is normal, little reaction is noted in the portal tract, and central bile stasis is present ($\times 125$). (Courtesy of Dr. P. Kimmelstiel.) E. Jaundice of 3 weeks' duration. Proliferation of ductules surrounded by inflammatory exudate ($\times 315$).

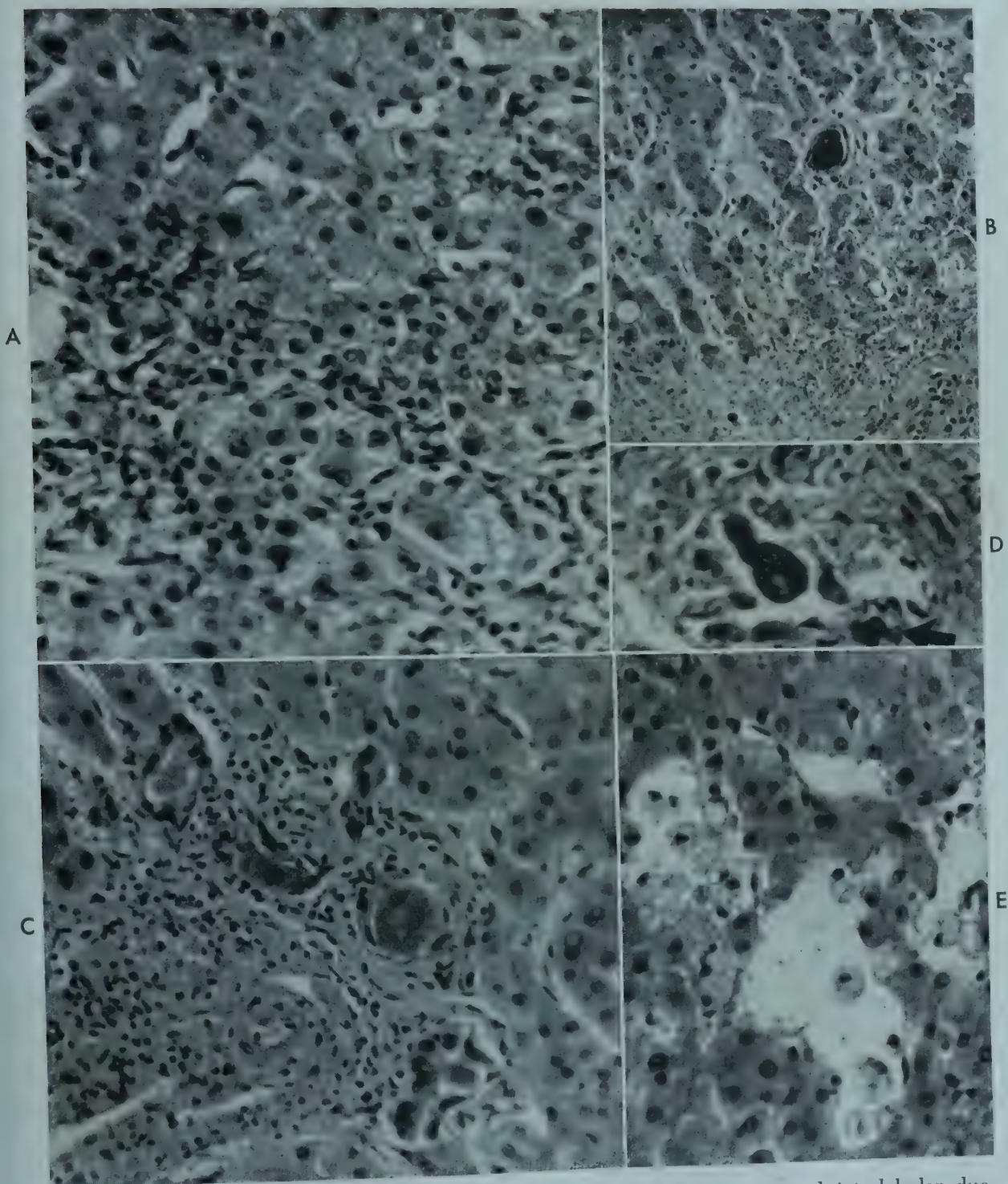


FIG. 98 A. Intrahepatic cholestasis of 4 weeks' duration. The perilobular and intralobular ductules are proliferated and surrounded by cellular exudate containing eosinophils. H&E ($\times 270$). B. Intralobular ductules filled with bile plugs in central hepatic necrosis (toxic hepatitis of unknown etiology). H&E ($\times 115$). C. Perilobular ductules filled with inspissated bile; the epithelial lining is compressed in some places and proliferated in others (fatal viral hepatitis). H&E ($\times 190$). D. Close-up of same, showing inspissated bile (indicated by arrow) below the epithelium of the ductule. Trichrome ($\times 360$). E. Small bile infarct near central vein in fatal viral hepatitis. The cytoplasm of the hepatic cells is rarefied, and the nuclei are pyknotic. H&E ($\times 230$).

their absence does not exclude hypersensitivity to drugs (see Allergic Cholangiolitis, Chap. 41). Variations in the intensity of the exudate depend on poorly understood factors. It appears less severe in post-mortem specimens than in biopsy specimens, sometimes surprisingly so (Fig. 97D). Intralobular accumulation of exudate cells may give the impression of a focal necrosis, but serial sections show that they actually accumulate around ductules.

Chronic Cholestasis. As exudate increases around proliferated periportal ductules, the lobular periphery may be destroyed (periportal necrosis) (Fig. 101D). Fibers eventually form around the intralobular and perilobular ductules, resulting in periductular fibrosis. This may strangulate the ductules. They are finally destroyed; in late stages of intrahepatic cholestasis, ductules are no longer found. In this stage of cholangiolitic or pericholangiolitic cirrhosis, the intrahepatic cholestasis, which originally was functional, has become mechanical, at least in part. The extrahepatic biliary tree is normal, and on exploration or cholangiography the bile ducts are seen to be narrow. The bile inspissated in the ductules sometimes forms bile sand in the extrahepatic biliary tract, with subsequent stone formation.

Extrahepatic Biliary Obstruction

In extrahepatic cholestasis the degree of obstruction influences the morphologic picture more than its duration. The more complete the obstruction, the more rapidly the structural changes appear.

Experimental Observations. Ligation of the common bile duct produces alterations which vary in different species, owing in part to variations in development of the gallbladder. Moreover, the site of ligation is of importance. In rabbits ligation of the hepatic duct results in more severe hepatic damage than ligation of the common duct, which permits filling of the gallbladder with relief of pressure [180]. The earliest change is a swelling of the cells in the immediate vicinity of the central vein. The cytoplasm contains granular condensation in the center of the lobule, with some of the granules showing bile pigmentation. The bile ducts and ductules are proliferated, and cellular infiltration of the portal tracts develops (Fig. 132, lower right). Between these cells, bile plugs are noted. Large areas of necrosis, termed "reticular necrosis," develop as early as 24 hours after common duct ligation in the rabbit and in the guinea

pig. In the guinea pig mainly the peripheral bile canaliculi are dilated. In rats, which have no gallbladder, ligation of the common duct near the hilus of the liver produces more severe changes than ligation of the common duct near the duodenum, which results in a cystic dilatation of the extrahepatic bile duct [1824].

Hepatic-cell Necrosis. Prolonged extrahepatic biliary obstruction produces changes in addition to those described under hepatic-cell degeneration and hepatic-cell necrosis in cholestasis generally. These occur after at least 3 weeks of complete biliary obstruction. In circumscribed lobular territories, mainly on the periphery and in the vicinity of portal tracts, the hepatic cells lose most of their cytoplasmic staining as an advanced stage of "feathery degeneration" (see Feathery Degeneration, under Hepatocellular Degeneration, Chap. 22). A loose reticulated network persists, or the cells appear almost empty. The network is imbibed with bile. The nuclei retain a central position, although they are pyknotic and finally disappear (Fig. 99C). The cells finally disintegrate and the framework collapses. Eventually only a fibrotic scar, almost devoid of cellular infiltration, remains. This bile infarct has been explained as a result of interference with circulation by the dilated bile ducts [2154]. Bile infarcts in extrahepatic biliary obstruction are much larger than in intrahepatic cholestasis and are found in the periportal area.

Bile Duct Dilatation. Dilatation of bile ducts in the liver, or hydrohepatosis, and dilatation outside the liver are found only in extrahepatic cholestasis (Fig. 100, upper and lower). The site of the obstruction determines the extent of the dilatation. In carcinoma at the bifurcation of the hepatic duct, the extrahepatic ducts are not dilated, whereas the entire biliary tree is dilated in carcinoma of the papilla of Vater or in stones or strictures in this location. Dilatation of bile ducts is not necessarily associated with jaundice. Obstruction of one branch of the hepatic duct, particularly by tumors, leads to dilatation of the ducts of the respective lobe, whereas the other lobes compensate in the excretion of bilirubin. In the dilated small bile ducts, bile plugs, called microoliths or microcalculi, form (Fig. 99A). Prolonged cholestasis (e.g., after 3 weeks of complete obstruction) leads to necrobiotic changes of the lining epithelium of the bile duct, with subsequent rupture and extravasation (Fig. 99B). The extravasated bile in the portal tract acts as an irritant

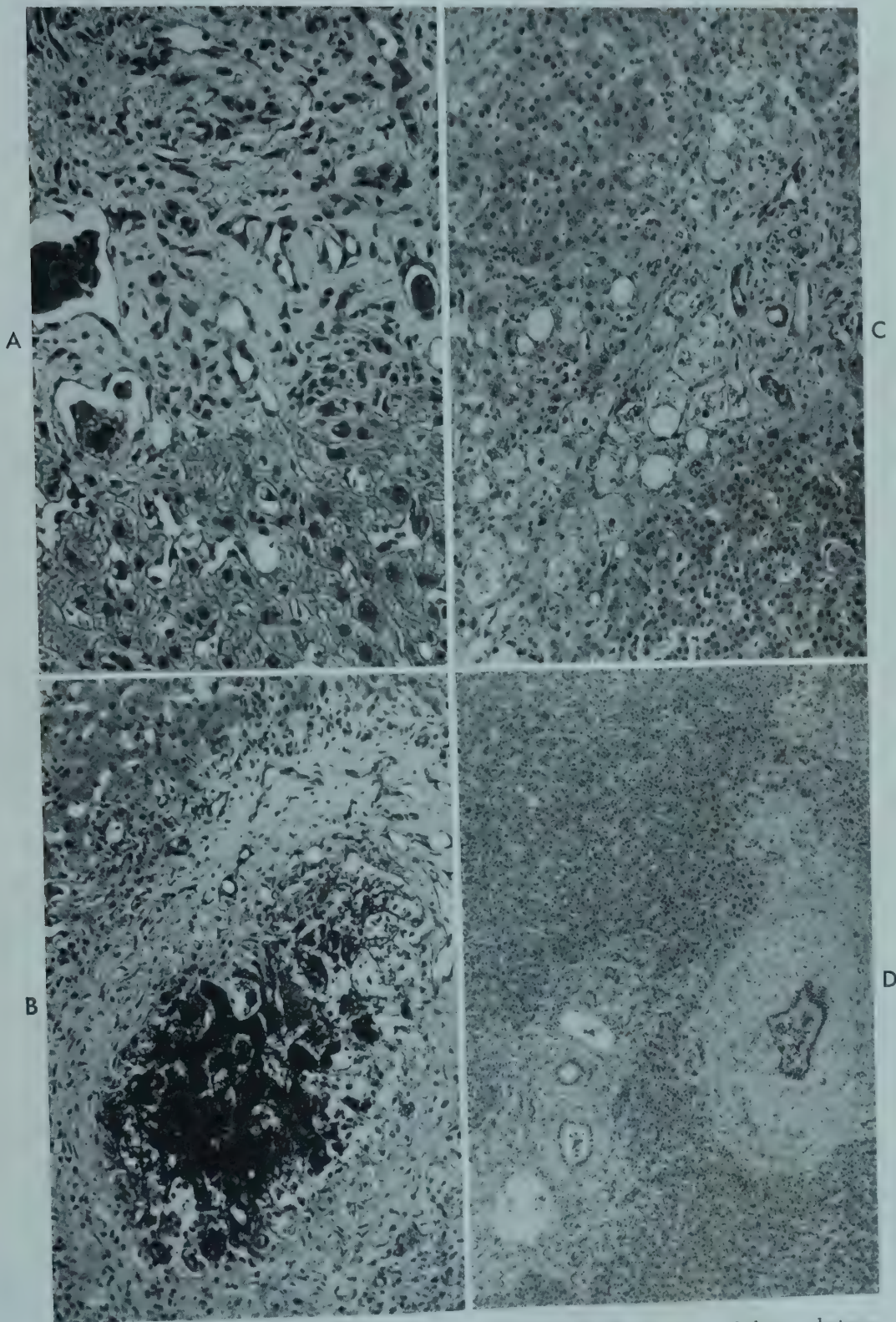


FIG. 99 Biopsy specimens of patients with prolonged extrahepatic biliary obstruction. H&E. A. Microcalculi in dilated ductules and ducts in the portal tracts ($\times 160$). B. Extravasate of bile from bile duct in portal tract, with destruction of the epithelial lining and accumulation of scavenger cells ($\times 110$). C. Bile infarct near portal tract; reticular appearance of bile-pigmented necrobiotic hepatic cells after loss of most of the cytoplasm ($\times 105$). (Popper, H.: *Am J Med.* 16:98, 1954.) D. Periductal scars persisting after relief of obstruction ($\times 40$).

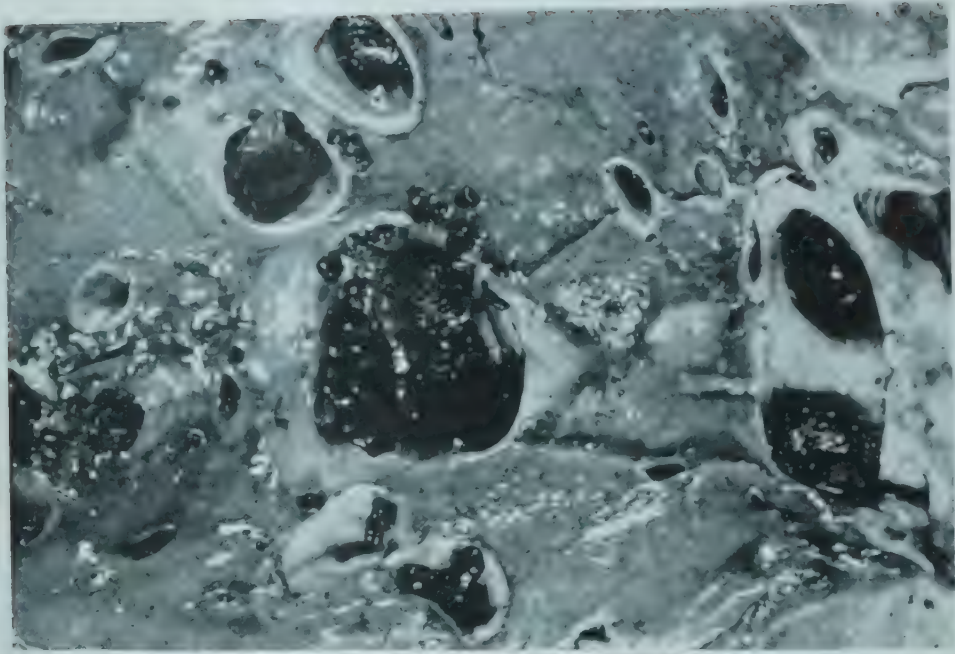


FIG. 100. *Upper.* Dilatation of the larger intrahepatic bile ducts (hydro hepatosis), caused by carcinoma at bifurcation of hepatic ducts. *Lower* Cholangiogram showing dilatation of extrahepatic bile ducts following relief of long-standing biliary obstruction caused by choledocholithiasis. (Courtesy of Dr. Lillian Donaldson)

and elicits a foreign-body reaction with giant cells and surrounding fibrosis, terminating in a scar containing large macrophages with golden-brown bile pigment. The dilatation of the biliary passages, particularly in the portal tracts and outside the liver, reflects the degree, duration, and the site of cholestasis more than any other morphologic alteration, and is a helpful diagnostic criterion in liver biopsies. Complete obstruction by tumors produces the severest changes, while the portal and extrahepatic biliary passages are spared in intrahepatic cholestasis. Bile extravasates in liver biopsy specimens prove the presence of extrahepatic obstruction.

Connective Tissue Alterations; Inflammation.

About 10 days after the onset of complete obstruction, alterations of the connective tissue patterns are noted [2802]. The collagenous fibers assume a circular arrangement, are thickened, and may possibly increase in number [2632] (Fig. 99D). Moreover, edema of the portal tracts develops, and they are eventually infiltrated by inflammatory cells, which also accumulate around perilobular ductules. This occurs even in the absence of bacterial invasion and is produced by the regurgitation of bile. Prolonged cholestasis produces periductular fibrosis, and periductular strands extend into the parenchyma from the enlarged and fibrosed portal tracts. The lobular periphery next to these scarred areas reveals bile stasis and pigmentation. The lobular architecture remains preserved for a considerable period of time, despite the periportal fibrosis. Eventually septums form in the forks of the perilobular strands as the result of the prolonged irritation. These subdivide the lobule, leading to cirrhosis formation (see Primary Septum Formation—Septal Cirrhosis, Chap. 28). Some have expressed doubt as to whether cirrhosis results from obstruction in the adult in the absence of infection [299, 1565]. In experimental animals, ligation of the hepatic duct produces cirrhosis [1160]. In human adults, however, complete obstruction is not tolerated long enough to produce a fully developed lesion, whereas incomplete obstruction, caused by stones or strictures, is frequently complicated by bacterial infection. Cirrhosis has been found most commonly associated with benign rather than with malignant obstruction [1666]. Noninfected biliary obstruction in congenital biliary atresia eventually causes cirrhosis [25]. The connective tissue reaction and inflammation in extrahepatic biliary obstruction justify the term "biliary hepatitis" (see Chap. 47,

Hepatic Injury from Extrahepatic Biliary Obstruction).

Bacterial Infection. Bacteria frequently settle in the portal tracts in the presence of extrahepatic cholestasis ("infected biliary hepatitis"). Infection more commonly follows benign rather than malignant obstruction. The bacteria responsible for the infection usually reach the liver by way of the portal blood stream. They pass through the sinusoidal wall and are drained toward the portal tract. Here inflammatory exudate accumulates around the lymphatic vessels. Ascending bacterial infection through the bile ducts is rare because of the bacteriostatic properties of bile as well as the flushing action of the bile flow. Even in the presence of bile fistulas, severe ascending infections are uncommon [324].

The infectious process is not uniform throughout all portal tracts. Morphologically, the infection is indicated by the presence of segmented leukocytes within the portal tracts around the bile ducts and occasionally within them (Fig. 101A). This leukocytic exudate also follows the ductules into the parenchyma (Fig. 101B, C). Small foci of polymorphonuclear leukocytes are thus scattered through the parenchyma, occasionally accompanied by focal necrosis. Eventually, periportal necrosis enlarges the edematous portal tract (Fig. 101D). The process may be acutely aggravated with the development of suppuration. Abscesses are located chiefly in the portal tracts and are usually connected primarily or secondarily with the bile duct (cholangitic abscesses) (Fig. 103D).

Fibrosis commonly follows or accompanies inflammatory and purulent exudation. It takes the form of scar formation in the portal tract, sometimes encircling the bile duct, and extends along the ductules into the parenchyma. The inflammation, independent of the ductular fibrosis, causes the formation of membranes and septums, which form fingerlike extensions and sometimes dissect the lobular parenchyma (Fig. 101E). The cirrhosis formation is more extensive than in noninfected cholestasis. The cirrhosis that is the end result of prolonged infected extrahepatic biliary obstruction is grossly characterized by pipestem-like scarring and widening of the portal tracts and has been called "secondary biliary cirrhosis" or "cholangitic cirrhosis" [2797]. It rarely follows primary cholangitis without extrahepatic biliary obstruction.

Recovery from Extrahepatic Biliary Obstruction. After relief of extrahepatic biliary obstruction.

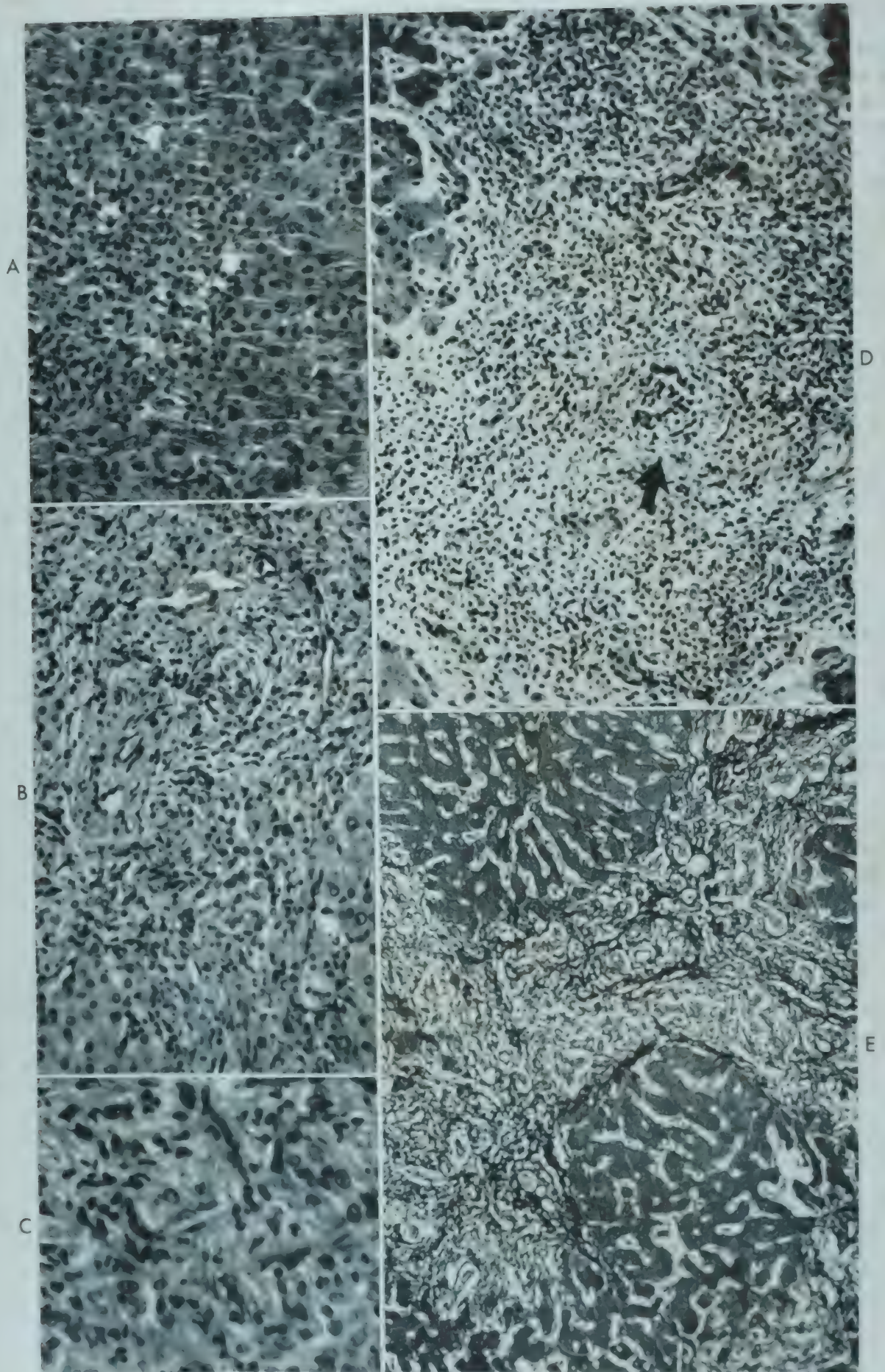


FIG. 161. Infected biliary obstruction. *A*, Accumulation of segmented leukocytes in small portal tract. H&E, $\times 145$. *B*, Increase of peribiliary ductules surrounded by leukocytic infiltrate in prolonged obstruction. H&E, $\times 145$. *C*, Close-up of *B* ($\times 235$). *D*, Periportal

tion, most histologic changes rapidly disappear. Degenerated and necrotic hepatic cells are replaced within a few days by rapid regeneration, starting from the periportal zone. Foci of feathery degeneration may persist for prolonged periods; in addition to irregularly shaped deposits of brown-pigmented material which coalesces, the deposits having been expelled from the hepatic-cell plates into the tissue spaces (Fig. 96, upper left). Small scars from collapse of framework are adjacent to the brown-pigmented material. Bile plugs in the center of the lobule persist for several weeks, and the hepatic cells, as well as the Kupffer cells, in this area contain a brown, finely granular pigment—modified bilirubin. The proliferation of the ductules also subsides, except in areas of perilobular fibrosis. Inflammatory changes in the portal tracts linger for some time, and eventually hyalinized collagen is deposited, resulting in a permanent slight enlargement of the portal tract.

Focal Mechanical Obstruction

Obstruction of one branch of the hepatic duct or of smaller branches is usually caused by benign or malignant tumors, abscesses, aneurysms of the hepatic artery, or parasites. Hyperbilirubinemia with generalized jaundice does not occur, since the nonobstructed portion of the liver excretes the excess bile. Local dilatation of bile ducts, with bile plugs in ductules and even local feathery degeneration, is noted. Such local changes are found even around small metastases and may thus become misleading in liver biopsy specimens.

CHANGES IN OTHER ORGANS

In prolonged biliary obstruction, changes in the function and structure of the myocardium, brain, and kidney occur. Osteoporosis occurs as a result of poor absorption of calcium because of the formation of calcium soaps in the intestine in the absence of bile salts.

FUNCTIONAL CONSEQUENCES

The biochemical consequences of cholestasis fall into four main groups, resulting from (1) regurgi-

tation of biliary products; (2) hepatocellular degeneration; (3) inflammation; (4) alterations of specific hepatic functions. They depend upon the duration and degree of cholestasis.

Regurgitation of Biliary Constituents

The rise of bilirubin and bile acids in serum is partially the result of regurgitation. Part of the bilirubin elevation is the result of inability of the hepatic cells to accept it. The sudden rise of bilirubin in hepatic failure in biliary obstruction results from faulty renal excretion, inability of the liver to accept any bilirubin, and a block of other pathways of pigment metabolism. Prompt-reacting bilirubin increases in the thoracic duct lymph following common duct ligation before the blood level is elevated [1220]. This phenomenon is less apparent with regard to alkaline phosphatase and is not seen at all with cholesterol. Injected Bromsulphalein rises rapidly in the thoracic duct lymph early in obstruction. The regurgitation of bilirubin results in bilirubinuria and absent or reduced fecal and urinary urobilinogen. The urine is dark, and the feces are clay colored.

The elevation of bile acids in the serum has been associated with pruritus. An indirect result of the diversion of bile acids by regurgitation is the disturbance in intestinal absorption. This inhibits vitamin K absorption and prolongs the prothrombin time, causing hemorrhagic tendencies (see Prothrombin Complex, under Clinical Manifestations of Hepatic Insufficiency, Chap. 23). The elevation of serum-phospholipid levels is possibly also caused by regurgitation [481]. In the dog, the histamine content of the blood rises soon after ligation of the common duct, possibly owing to regurgitation of biliary histamine [83]. In general, the more complete the obstruction of the bile flow, the more regurgitation occurs. In experimental animals, the closer the ligation of the common duct to the liver, the greater the rise of bilirubin in the blood [1824]. In man, extrahepatic removal mechanisms for some of these regurgitated substances are not so well developed as in animals, and substances such as Bromsulphalein may remain in the blood stream for long periods of time in extrahepatic biliary obstruction [1163].

necrosis and extensive leukocytic infiltration of enlarged portal tract, with leukocytes in interlobular bile duct (arrow) in prolonged obstruction (transition into cholangitic cirrhosis). H&E ($\times 115$). E. Perilobular fibrosis with fingerlike extension around lobules and beginning dissection of lobules in secondary biliary (cholangitic) cirrhosis. Mallory's aniline blue ($\times 52$).

Hepatocellular Degeneration

The duration and degree of cholestasis determine the extent of the changes which result from associated hepatocellular degeneration. The serum-albumin level is reduced and the globulin level is elevated in 50 per cent of cases [2277], cholinesterase level is reduced in 40 per cent [2640], galactose tolerance is impaired in 25 per cent, and hippuric acid synthesis is impaired in 82 per cent [2640]. Although the cholesterol ester ratio is low in only 40 per cent of cases, it is a sensitive indicator of hepatic-cell degeneration in cholestasis [1760, 2195]. When bacterial infection complicates cholestasis, hepatic-cell degeneration is aggravated independently of the degree and duration of the cholestasis [2277]. The hepatic esterase level is greatly reduced, while alkaline phosphatase activity is increased, particularly after high common duct ligation [1824]. Hepatic and plasma-vitamin A levels are low [2625]. Serum-histamine levels are increased and seem to be related to the degree of pruritus [2316].

Inflammation

Inflammation leads to elevation of the serum-gamma globulin level. This effect is less outspoken in cholestasis than in other types of hepatic injury probably because of impaired gamma globulin formation by the bile-laden Kupffer cells (see Inflammation, under Morphologic Appearance, earlier in this chapter).

Specific Alterations of Hepatic Function

The phenomena described above do not completely account for the elevated levels of phospholipids and cholesterol and for the increased alkaline phosphatase activity in cholestasis. Altered function caused by a specific stimulus must be assumed, since these changes are not well correlated with the degree and duration of the obstruction. Although extreme changes are found occasionally in extrahepatic biliary obstruction, they are usually more outspoken in intrahepatic cholestasis. In general, phospholipid level and alkaline phosphatase activity are parallel, while cholesterol varies independently.

PHOSPHOLIPIDS. In tracer studies with radioactive phosphorus, the specific activity of the phospholipid phosphorus in cholestasis remains constant, despite the increase in serum phospholipid [147]. This is interpreted as showing an increased

rate of formation of this substance. Comparison of specific activities of plasma and biliary phospholipids indicates that early in cholestasis the slight elevation of phospholipids is caused by regurgitation, but high levels must be the result of new formation [2904].

CHOLESTEROL. The elevation of the serum-cholesterol level in biliary obstruction [37] can not be explained by mere regurgitation or retention of cholesterol normally excreted in the bile or by alteration of intestinal excretion or absorption of cholesterol. Excessive formation has been demonstrated with radioactive carbon and tritium in animals with ligated common ducts [1084]. Some of the increased serum cholesterol may be the result of increased bile acids in the blood [454], since injection of cholates raises the serum-cholesterol level [1100]. This may be caused by greater solubility of cholesterol, since detergents also raise the serum level [1100]. In general cholesterol and phospholipid levels rise simultaneously in cholestasis, along with an increase in beta globulin [1876]. A specific lipoprotein may be formed [882]. The phospholipid elevation is of greater magnitude than the total cholesterol elevation [37, 147], apparently because the phospholipid level parallels the free cholesterol level rather than the total cholesterol level [37]. In view of the stabilizing action of phospholipid, elevated phospholipid levels may increase the solubility of cholesterol, or elevated cholesterol levels may stimulate formation of phospholipids to keep them in solution. The parallel increase of serum-cholesterol and phospholipid usually associated with a slight increase in neutral fat raises the total lipid level. At lipid levels above 1,800 mg per 100 ml, skin xanthomas develop owing to the deposition of lipids in the connective tissue [26]. This occurs in chronic intrahepatic cholestasis, xanthomatous biliary cirrhosis [266, 2156], occasionally in infectious hepatitis [955], arsenic hepatic injury [26, 3236], experimental hepatic injury, and in extrahepatic biliary obstruction caused by tumors [1439], strictures [140], congenital bile duct atresia [25], or in experimental bile duct ligation [3537]. The xanthomas disappear after the serum-lipid levels fall. Patients with total lipid levels between 1,300 and 2,000 mg per 100 ml show xanthelasma, while those whose levels are below 1,300 mg per 100 ml show no deposition of lipids in the tissues [26, 27]. Despite the high total lipid level, the serum is often

ear, and atherosclerosis is not necessarily a complication. Both facts are explained by the relatively high phospholipid level.

ALKALINE PHOSPHATASE. Increase in serum-alkaline phosphatase activity in cholestasis has not been fully explained (see Serum Alkaline Phosphatase, Chap. 7). Phosphatase elevation and bilirubinuria are not parallel, and phosphatase is said to be less readily excreted in the bile than bilirubin, thus explaining its rise in the absence of jaundice. Regurgitation or retention does not suffice as an explanation, and therefore altered formation is at least suggested.

Serum-hyaluronidase inhibitor, somewhat like serum-alkaline phosphatase activity, is increased in obstruction and reduced in hepatic failure [3121].

DEPRESSION FACTOR. Another phenomenon characteristic of cholestasis is the presence of a factor in serum that depresses turbidity in the zinc sulfate- or thymol-turbidity tests and possibly also in the cephalin-flocculation test [2636]. This depression factor stabilizes the serum proteins in conditions which would otherwise tend to increase flocculation or turbidity. It is possibly related to lecithin and has great diagnostic sig-

nificance [2636] (see Zinc Sulfate-turbidity Test and Thymol-turbidity Test, Chap. 33).

Clinical Manifestations

Unlike hepatocellular degeneration, cholestasis, as such, produces few symptoms, despite deep jaundice. Anorexia and malaise are minimal, and even weight loss may not be striking. In later stages, these symptoms appear because of complicating hepatocellular degeneration. The main clinical symptom characteristic of all stages of cholestasis is severe itching, which can be intolerable. Excoriations are usually hemorrhagic because of bleeding tendencies. For symptomatic relief, cortisone, methyltestosterone, adenosine-monophosphate, antihistamines, and intravenous procaine have been recommended [27, 2038].

Biliary obstruction, even if complete as a result of an extrahepatic mechanical cause, can be tolerated for a surprisingly long time, especially in children, in whom it may persist for several years. Adults usually succumb from the cause of the obstruction, such as carcinoma. Palliative surgery, with formation of internal or external fistulas, is indicated only to control or prevent symptoms such as itching or bleeding tendencies.

Since the epithelial structures predominate in the liver and the response to injury leads to reaction by the epithelium, inflammatory changes are less important than hepatic-cell degeneration from a functional standpoint. Nevertheless milder inflammatory changes are common. Much of the inflammatory reaction is probably a response to the hepatic-cell degeneration, rather than a primary process. For instance, in focal necrosis the mesenchymal reaction and cellular infiltration may be morphologically impressive, while functionally, the loss of hepatic cells is the important factor. Similarly, diffuse Kupffer cell mobilization associated with hepatic-cell degeneration is usually reactive rather than primary. Some histologic signs of hepatic inflammation are found in biopsy specimens of almost every adult liver. Inflammatory reactions which are regularly found in hepatic diseases are often considered characteristic for the disease. Similar histologic features may be seen, however, in the absence of symptoms or laboratory findings attributable to the liver. Great caution is therefore required in the interpretation of histologic findings of inflammation. The following are the signs of inflammation found in the liver: (1) edema; (2) Kupffer cell mobilization; (3) foci of inflammatory cells; (4) portal inflammation due chiefly to perilymphangitis; (5) pericholangiolitis; (6) cholangitis. These signs may occur alone or in any combination.

Edema

Separation of the sinusoidal wall from the hepatic-cell plates with opening of the Disse spaces and filling of these spaces with albuminoid material in fixed-tissue sections is referred to as *hepatic edema* (Fig. 32).

Etiologic Factors. Some edema develops in the premortal, agonal period, and therefore its occurrence in autopsy specimens does not necessarily indicate its existence before the terminal period [2625, 3629] (see Agonal and Postmortal Changes, Chap. 20). More severe degrees, however, in which the sinusoids appear compressed by the interstitial edema, are more than a premortal phenomenon [1732]. This severe edema occurs in a variety of conditions, some of which are probably merely mechanical and the result of increased venous pressure. Others are the result of alterations of the capillary wall. This results from anoxia or from injurious substances and, therefore, can be considered exudative or inflammatory in nature. In the latter form, increased permeability of the sinusoidal wall for serum proteins is important [2280] and is reflected in an increased flow of protein-rich lymph [945]. The term "serous hepatitis" [945, 2797] has been applied to this condition, although it is not necessarily associated with the presence of exudate cells. Other factors, such as the retention of sodium or excessive release of tissue proteins from damaged hepatic cells into the perisinusoidal spaces, may share in the formation of edema [894]. Edema resulting in congestion is usually more apparent in the centrilobular portion, while edema owing to disturbed permeability, as found in infections and intoxications, is diffuse. Malnutrition has also been considered a cause of diffuse hepatic edema [1491].

Structural Changes. Anatomically, severe intra lobular edema is associated with a grossly visible widening of the portal spaces and thickening of the gallbladder bed, since the lymphatic vessels of the gallbladder are in communication with the lymphatic vessels of the liver within the

portal tracts [945]. Grossly, the liver under such circumstances is enlarged, and the edges are blunted. Edema is not more common in hepatic diseases, with the exception of toxic hepatitis and congestion, than in nonhepatic diseases.

Functional Significance. Theories that have ascribed great significance to hepatic edema in the morphogenesis of various hepatic disorders, including viral hepatitis, find little support from present evidence. Little is established about functional impairment specifically related to edema, despite the impressive anatomical picture [2458]. Nevertheless, various claims have been made. For instance, toxic edema, or serous hepatitis, has been said to interfere with the metabolism of the hepatic cells [945, 2797]. Organization of the protein-rich exudate in the Disse spaces has been said to cause interstitial fibrosis and eventually cirrhosis [1491]. Experimentally, hepatic edema has been produced by histamine in dogs [945] and by urethane and allyl formate in rats [810, 2832]. However, extravasation of red cells rapidly follows the edematous stage in such instances.

Proliferation of Kupffer Cells and Portal Histiocytes

Increase in the number of Kupffer cells may be caused either by transformation of resting endothelial cells into active cells engaged in phagocytosis, or by new formation of active cells. Histiocytes that often appear in the portal tracts are wandering reticuloendothelial cells related to and often derived from Kupffer cells. They are also engaged in phagocytosis. Some proliferation or mobilization of the Kupffer cells and portal histiocytes is found under normal circumstances (see next section, Focal Necrosis), and the line between normal and abnormal activity is difficult to draw. This reaction accompanies many nonhepatic diseases and is especially prominent if the reticuloendothelial cells engulf abnormal circulating material, such as bacteria in subacute bacterial endocarditis. In liver diseases the cells are mobilized apparently because they engulf hepatic-cell breakdown products, and much pigment can be seen in them, usually giving a PAS reaction but seldom containing iron.

Structural Changes. The cytoplasm of enlarged Kupffer cells, focally or throughout the liver, contains many basophilic bodies which merge under low magnification to make the basophilia appear diffuse. In other instances, such

as typhoid fever, the cytoplasm appears diffusely basophilic. In addition, the cytoplasm contains granular pigmented and nonpigmented engulfed material or lipids, indicated by vacuoles (Fig. 34, upper left). The nuclei become vesicular and large. Almost all endothelial cells can assume the shape of the bulging Kupffer cells; only a few remain flat. The Kupffer cells extend into the sinusoidal lumen, and in some sections appear to be floating in the lumen, apparently an artefact [907]. The enlarged and mobilized Kupffer cells sometimes become very large and appear like giant cells, especially if they are crowded with microorganisms, as in histoplasmosis (Fig. 183D) or kala-azar (Fig. 183E). Very large Kupffer cells, sometimes completely free in the sinusoids, have engulfed lipid material. Their cytoplasm appears diffusely eosinophilic in routine stains, but either PAS or fat stains are strongly positive. This is noted in lymphomas or after amniotic fluid embolism.

Functional Significance. Kupffer cell mobilization is usually associated with increased serum-gamma globulin formation. This serum protein is a specific product of the reticuloendothelial system as a whole and, in this instance, of the Kupffer cells. The relation of excessive gamma globulin production to antibody formation has been discussed (see Antibody Formation, under Kupffer Cells as Part of the Reticuloendothelial System, Chap. 14).

Specific Changes. Kupffer cell mobilization is especially severe in salmonella infections, such as typhoid fever; in granulomas such as tuberculosis or syphilis where the granuloma seems to originate from the Kupffer cells; or in Hodgkin's disease. The excessively mobilized Kupffer cells in these instances obstruct the sinusoidal lumen and cause focal necrosis of hepatic cells and intralobular granulomas. Fibrosis may be the end stage, probably owing to transformation of the proliferated histiocytic cells, including the Kupffer cells, to fibroblasts. Whether Kupffer cell mobilization without granuloma formation is followed by transformation of these cells into fibroblasts with the development of fibrosis is not established.

Focal Necrosis; Intralobular Granuloma

Necrosis of single hepatic cells or groups of cells usually attracts scavenger cells, which take the place of the necrotic and disappearing hepatic cells, so as to produce an accumulation of these

cells (focal necrosis) (see Focal Necrosis, under Necrosis, Chap. 22). The foci vary in size from a few cells to large areas of necrosis involving a large part of the lobule (Fig. 89D, E). The nature of the scavenger cell varies; in general, polymorphonuclear leukocytes predominate. In viral infections, particularly viral hepatitis, mononuclear cells are in the foreground—first histiocytes, later plasma cells (see Structural Alterations, Chap. 43). In most cases the stroma is not altered except for rare instances of fibrinoid necrosis, which can be experimentally produced by hyperergic reactions [1124].

Eventually cellular accumulations from any cause appear like small granulomas; then they can not be differentiated from primary Kupffer cell mobilization or from infiltration occurring around intralobular ductules. Even large or densely arranged areas of focal necrosis do not cause functional alterations if the surrounding cells are normal. Small areas of nonspecific focal necrosis are exceedingly common and are found in biopsy specimens in the absence of any clinical findings in otherwise normal livers. In surgical biopsy specimens, they result from the operation itself [1715].

Portal and Periportal Inflammation

Some migrating cells are found in almost every portal tract of the adult liver. The portal tracts are sometimes extensively infiltrated by lymphocytes, histiocytes, and occasionally segmented neutrophilic leukocytes. Significance can be attached to this only if all or most portal tracts of the liver contain accumulations of inflammatory cells. Even where the infiltration is dense, its relation to the structures of the portal tracts is not clear (Fig. 102A).

Morphogenesis and Localization. The nomenclature of portal inflammation is confused; it can best be defined in terms of location and pathogenesis. Noncommittal terms such as "periacinose hepatitis" [943] or "triaditis" [3549] avoid reference to specific structures. In view of the proximity of the bile ductules and lymphatic vessels in and around the smaller portal tracts, whether the lesion is perilymphangitic or pericholangiolitic is difficult to decide. Exceptionally a primary damage of the connective tissue with fibrinoid degeneration occurs.

PERICHOLANGIOLITIS. This assumes that portal inflammation is related to the intralobular or periportal bile ductules or to the smallest bile

ducts in the tracts. This is supported by the presence of inflammatory cells, especially segmented leukocytes, in the walls and lumens of the small ducts. However, such cellular exudate may result from invasion of the bile ducts from the outside. Functionally, no evidence exists for involvement of the biliary tree in most cases; therefore a cholangiolitic origin can be discarded, except in instances of intrahepatic cholestasis, or "cholangiolitis" (see Cholangiolitis, later in this chapter).

PERILYMPHANGITIS. Morphologically, inflammatory cells often accumulate around preformed spaces, such as the smallest lymphatic vessels in the portal tracts (Fig. 103A). These spaces are frequently areas of lymphangectasia. Irritating material which has reached the liver via the portal blood flow is drained through the interstitial spaces to the lymphatic vessels in the portal tracts and to a lesser degree in the central fields. The endothelial lining of the lymphatic vessels engulfs the irritating material, setting up an inflammatory process at these sites [1996]. Portal inflammations are mostly perilymphangitic. The importance of the lymphatic vessels in hepatic disease and inflammation has long been recognized. Exudative lymphangitis was separated from productive lymphangitis [1996, 2046, 3599], and emphasis was placed on inflammation originating in the gallbladder [1243]. Lymph vessels probably extend into the intralobular trabeculae, which contain arterioles and ductules [907], and some intralobular accumulations of inflammatory cells arranged in the form of strands are perilymphangitic.

PERIportal INFLAMMATION. The cellular inflammatory exudate may extend from the portal tracts into the periportal parenchyma, usually in the form of streaks. These consist of lymphocytes and histiocytes or segmented leukocytes, depending on the character of the exudation in the portal tract itself. This exudate is also associated with disruption and disorganization of the limiting plate and with necrosis of the hepatic cells on the periphery of the lobule (Fig. 103C). It therefore indicates periportal or peripheral necrosis morphologically (see Peripheral Necrosis, under Necrosis, Chap. 22) but has the significance of portal infiltration etiologically.

Etiologic Factors. Injurious substances from the intestine, bacterial or otherwise, probably cause the commonly encountered portal inflammations which usually involve only some fields and spaces

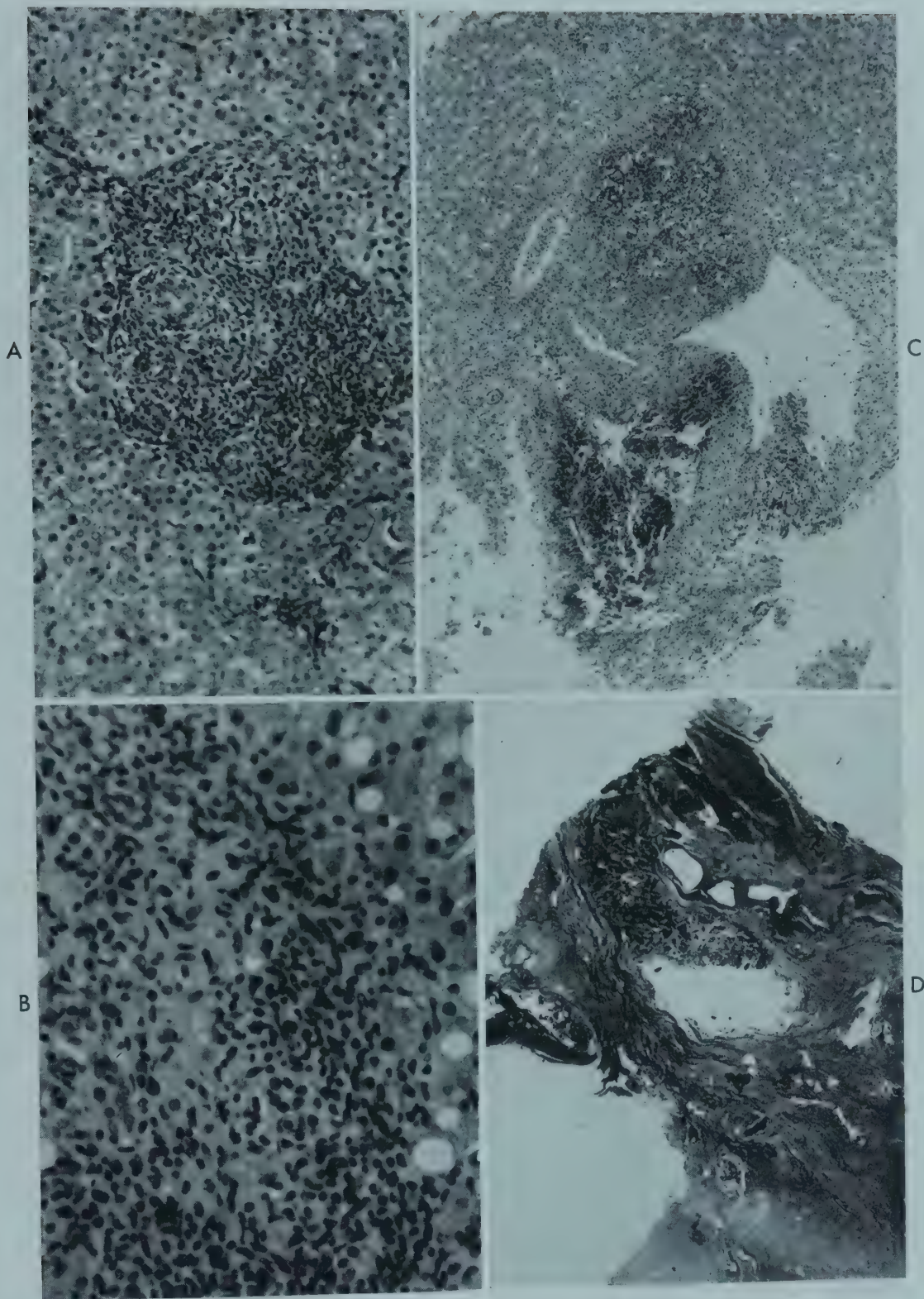


FIG. 102 A. Accumulation of segmented leukocytes in the slightly widened portal tracts extending into the lobular parenchyma along ductules in a patient with pneumonia. H&E ($\times 115$). B. Accumulation of lymphocytes and round cells in portal tract in a patient with a peptic ulcer. H&E ($\times 230$). C. Purulent exudate in dilated bile duct and in the surrounding tissue of the portal tracts extending into the parenchyma in choledocholithiasis. H&E ($\times 37$). D. Scarring around dilated main branch of hepatic duct in stricture of the extrahepatic portion of the hepatic duct ($\times 3$).

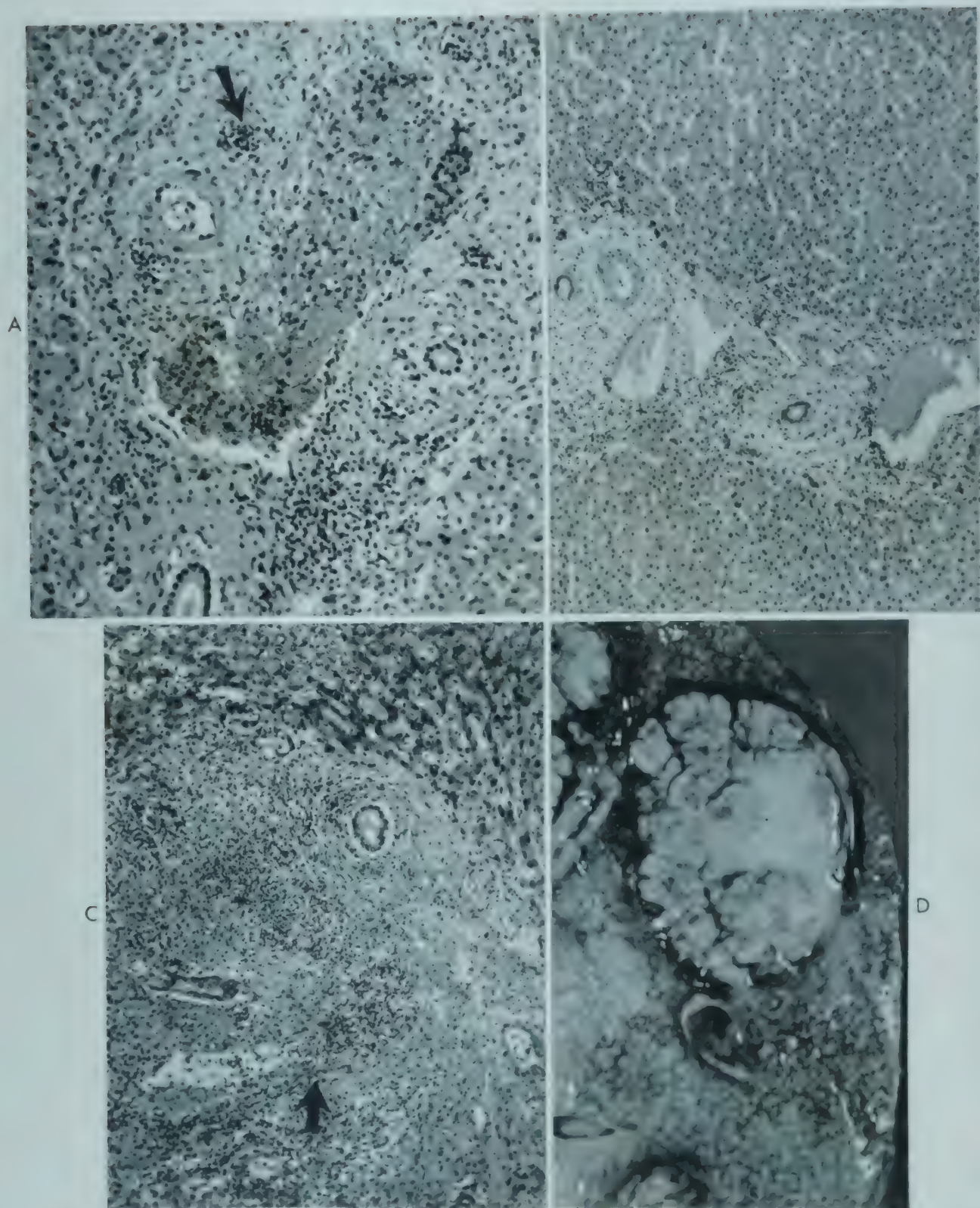


FIG. 103 A. Hepatic lymphangitis (arrow) in portal vein thrombosis following pancreatic abscess. H&E ($\times 120$). B. Scarring of portal tract, apparently as a result of preceding portal inflammation. Incidental finding without clinical symptoms referable to the liver. H&E ($\times 50$). C. Suppurative endophlebitis in portal vein branch (arrow) with beginning pylephlebitic abscess in chronic cholelithiasis. H&E ($\times 80$). D. Pylephlebitic abscess in suppurative diverticulitis.

most others. Therefore, any process which influences the permeability of the intestinal wall, such as anesthesia, can produce such a lesion. This seems to be the etiology of the portal infiltrations in otherwise normal livers. They also may be remnants of a clinical or subclinical attack of viral hepatitis. Such lesions are found in persons dying instantly in accidents who had no known disease [2626], or in autopsy material in diseases not primarily hepatic [2450]. They have been related to cholecystitis [2450] but are not specific. In almost every type of infection or bacteremia, perilymphangitic portal infiltration is noted. It is most severe in enteric infections such as cholecystitis [2244], colitis [1753], appendicitis, and tuberculous enteritis [1735]. This lesion also appears in almost any form of intralobular hepatic injury independently of its etiology and is then caused either by hepatocellular breakdown products or by injurious substances ordinarily detoxified by the hepatic cells.

Structural Changes. The appearance depends on the acuity of the process as well as its cause.

NONSPECIFIC FORMS. The most common form of portal inflammation shows accumulation of exudate cells, with predominance of lymphocytes and histiocytes and only a few segmented leukocytes (Fig. 102B). Sometimes many lymphocytes accumulate in a nodular form and are even arranged around a central germinative center called "benign hepatic lymphoma" [1735]. These are insignificant and do not reflect the status of the lymphatic tissue in the rest of the body. In acute bacterial infections, particularly in the portal venous system, segmented leukocytes sometimes aggregate to a degree, suggesting the term "purulent hepatitis." This is indistinguishable from a pylephlebitic abscess (Fig. 103D), which finally results. In all forms an increased number of perilobular ductules is common.

SPECIFIC FORMS. Portal and periportal infiltrations are found in acute viral hepatitis [1714, 2189] and may be the only visible lesion in its chronic stage [125, 1060, 2711, 2801], the exudate varying cytologically from that in other hepatic injuries (see Persistent Portal and Periportal Changes, under Types of Protracted Hepatitis, Chap. 44).

In granulomatous diseases, the portal infiltrations are characteristically distinct, as in tuberculosis (Fig. 179C), brucellosis (Fig. 181, upper right), and typhoid fever. In chronic lymphocytic leukemia, the leukemic infiltration is localized in

the same area as is the granuloma in Hodgkin's disease (Fig. 203, middle left).

Functional Significance. Portal inflammation frequently has no functional significance. It is correlated statistically with elevation of the sedimentation rate [1074]. The serum-gamma globulin level is often elevated, owing to excess formation by reticuloendothelial elements in the portal tracts.

Sequelae. The sequelae of the portal inflammation are unpredictable from its anatomic appearance. Fibroplasia commonly occurs, and scarring is a frequent and often innocuous end result (Fig. 103B). Fiber and membrane formation in the lobular parenchyma is found, however, in some instances and is the initial stage of portal cirrhosis, which progresses to dissection of the lobular parenchyma. In view of the ubiquitous character of the lesion, the often banal pathogenesis, and the unsolved problem of prognosis, it is advantageous to avoid designations such as portal [1996] or infiltrative [2244] hepatitis.

Cholangiolitis

The existence of inflammation in and around the smallest bile ducts and ductules has intrigued clinicians and pathologists for many years. This inflammation can be separated from perilymphangitis only with difficulty. It has served as the explanation of conditions with varying symptomatology but basically characterized by intrahepatic cholestasis. The inflammation in most instances appears to be secondary to increased permeability of the ductules and has been discussed under cholestasis (see Intrahepatic Cholestasis, Chap. 24).

Bacterial Cholangiolitis. A few instances of bacterial cholangiolitis have been reported [3070]. The bacterial infection was primarily localized in the ductules, the smaller bile ducts, and in some instances even in the larger bile ducts. The claim has been made that various infectious diseases such as pneumonia, influenza, and others may be complicated by low-grade cholangitis and cholangiolitis resulting in obstruction—"chronic obliterating cholangitis" [1801]. By analogy with subacute bacterial endocarditis, the presence of *Streptococcus viridans* led to the term "cholangitis lenta." This was held responsible for protracted biliary obstruction and was thought to terminate in cholangitic cirrhosis [943, 1903, 2156, 3510]. In recent years rare instances of hematogenous bacterial lesions have been described [137, 1801, 2560]. A bacterial etiology of these lesions is sup-

ported by the occasional dramatic response to antibiotics [500, 525] or sulfonamides. [2686], although the incidence and significance of bacterial cholangitis are not established.

Intrahepatic Purulent Cholangitis

This lesion is much more clearly defined than cholangiolitis, in that purulent exudate is seen in the lumen of the intrahepatic bile ducts. Only exceptionally are the ductules involved.

Primary Form. Bacterial infections have been claimed to be the cause of descending cholangitis without preceding involvement of other parts of the biliary tract. Such rare primary cholangitis was considered to be either enterogenous from the portal vein or hematogenous from the hepatic artery [3388]. Hematogenous infections arise from bacteria discharged from various foci of infection in the body [1913]. Despite the fairly extensive earlier literature, there has been little mention in recent years of primary, or systemic, cholangitis.

Secondary Form. The most common form of suppurative cholangitis is secondary to involvement of the biliary tract. It is associated with obstructive lesions or, less commonly, with external or internal bile fistulas. Occasionally, nonobstructive suppuration, such as that from cholecystitis, or even more remote infections in the portal system, such as appendicitis, may lead to cholangitis. Spontaneous rather than surgical bile fistulas are associated with cholangitis. Cholangitis has been produced in experimental animals by ligation of the duct simultaneously with installation of bacteria, or by anastomosing the common duct to the duodenum [1910] or to a long, blind, antiperistaltic loop of jejunum [1800]. Cholangitis produced in this way is often associated with cholecystitis and biliary cirrhosis, especially after the jejunal anastomosis. If surgical strictures are produced with the sphincter of Oddi intact, only minimal cholangitis results, although the ducts become greatly dilated.

Pathogenesis. Incompletely obstructing lesions in the common or hepatic duct, chiefly stones or strictures, or occasionally tumors of the papilla of Vater [899], are common causes of human cholangitis. Originally, infection ascending from the intestine was considered to be the only source of this cholangitis. While this is true for many cases, stasis alone may facilitate the settling of bacteria if they should be excreted into a slowly moving bile after their removal from the portal circulation.

Therefore, some instances of cholangitis develop from descending rather than from ascending infections. Suppurative cholangitis secondary to a liver abscess is always descending. It may develop even from abscesses that are not primarily cholangitic but are pylephlebitic ones which have secondarily broken into a bile duct.

Etiology. The vast majority of the suppurative lesions are due to bacteria. The most common offender is *Escherichia coli*, followed by *Aerobacter aerogenes*, *Streptococcus faecalis*, and *Bacillus proteus* [70]. Other types of bacteria are rare [2849]. These bacteriologic variations have become of great importance in view of antibiotic therapy. For the development of the suppurative form, either bile stasis with bacteria or, in the absence of stasis, overwhelming bacterial infection is required, since excretion of bacteria in the bile (bacteriocholie), as such, does not necessarily lead to cholangitis, as shown in animal experiments. Parasitic infestations, such as ascariasis, may lead to obstruction with ascending cholangitis. Regurgitation of pancreatic juice produces cholangitic irritation, but this seems to be more a theoretical possibility than an actual one.

Structural Changes. Histologically, segmented leukocytes are seen migrating through the epithelium, which is sloughed off in some areas. The presence of an occasional leukocyte between the epithelial cells is not necessarily abnormal. The lumen of the duct is filled by mucopurulent material, which is also found in the adnexal sacculae of the larger ducts. The tissue around the duct is infiltrated by pus cells (Fig. 102C). With subsidence of the process, circular scarring indicates healed cholangitis (Fig. 102D). The tenacious character of the intraluminal exudate in the acute and subacute stages produces temporary obstruction, with clinical symptoms, and also facilitates retrograde infection. The end result is the formation of a cholangitic abscess (Fig. 104, bottom). These abscesses may be small and multiple or large and single. The origin from cholangitis is not always clear, and the differentiation from pylephlebitic abscesses may be difficult. The golden-brown color of the pus suggests a cholangitic origin. Perforation through the diaphragm into the lung is more common with cholangitic and amebic than with pylephlebitic abscesses. Scarring following diffuse cholangitis leads to a diffuse thickening of the biliary ductal system which is grossly visible and which is called "pipestem cholangitic cirrhosis."

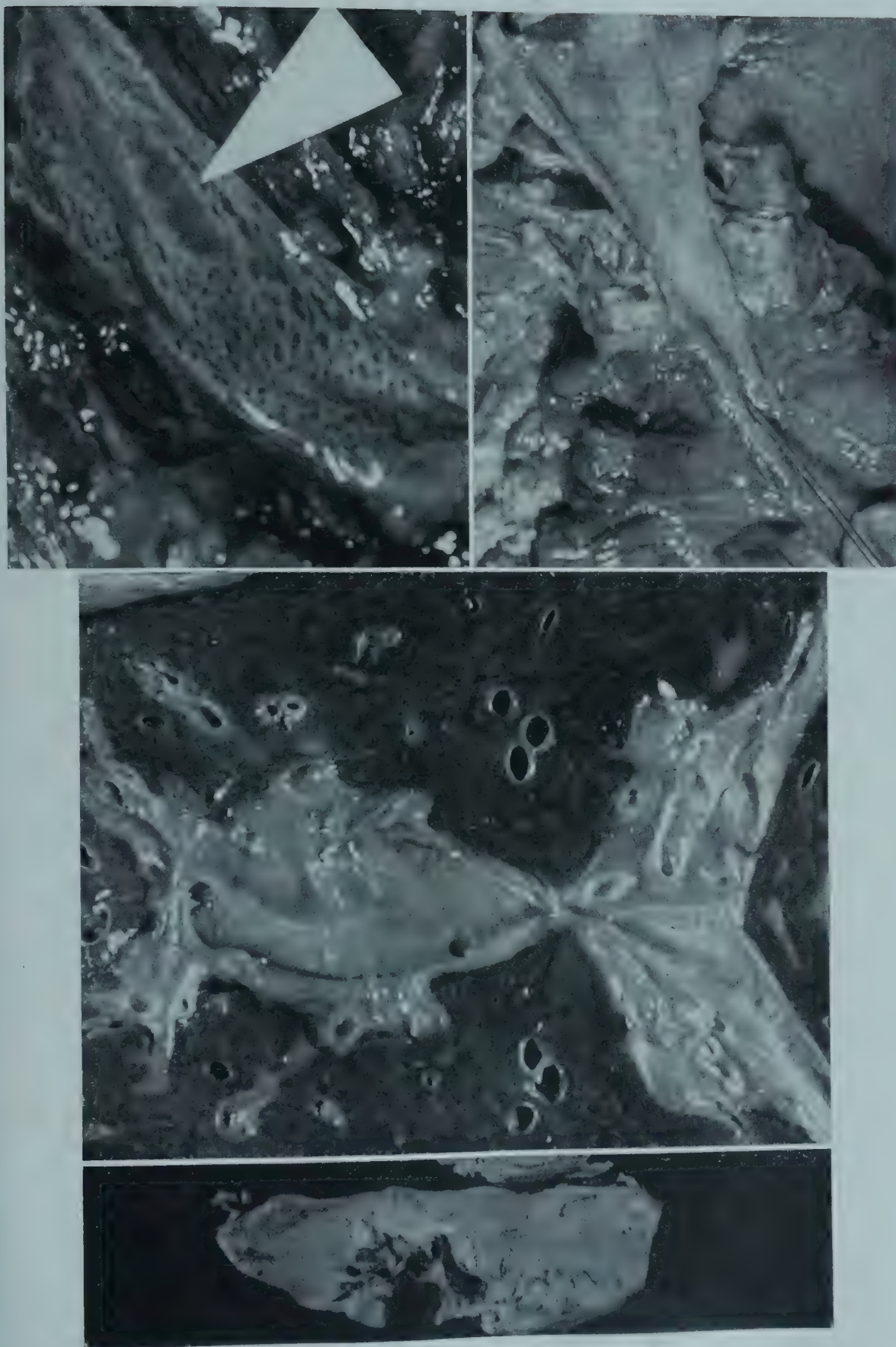


FIG. 104 *Top left.* Decubital ulcer indicated by arrow in common duct produced by stone. *Top right.* Probe pointing to stricture in lower portion of the common duct; the duct proximal to the stricture is dilated. *Center.* Stricture at bifurcation of hepatic duct in hepatolithiasis and choledocholithiasis. *Bottom.* Cholangitic abscess near the hilus of the liver in prolonged cholechololithiasis.

Functional Changes. Suppurative cholangitis is characterized by jaundice with cholestasis, enhanced periodically by obstruction of the biliary tree by viscid material. During this obstruction, the temperature rises, producing the spiking Charcot fever. The leukocyte count also spikes, reaching levels of 30,000, sometimes returning almost to normal within hours. During periods of obstruction the liver is very large and extremely tender. The spleen is permanently enlarged. Because of the associated hepatic-cell damage, the results of tests which indicate hepatocellular degeneration, including the flocculation tests, and even zinc sulfate turbidity, are abnormal.

Extrahepatic Cholangitis

This may occur as an acute nonsuppurative, or catarrhal, cholangitis, suppurative cholangitis, or cicatrizing cholangitis.

Nonsuppurative Cholangitis. The existence of a nonsuppurative inflammation, either choledochitis or inflammation of the papilla of Vater, is poorly substantiated anatomically. For almost a century claims for the existence of such lesions have been presented, primarily on the basis of clinical observations. Virchow considered catarrhal jaundice to result from catarrhal papillitis as an extension of a duodenitis. Whether a mucous plug can produce a biliary obstruction against the secretion pressure of the liver is questionable. In the French literature the importance of such a lesion is emphasized, the view being that it takes part in the production

of clinical manifestations by altering motility of the duodenal wall and interfering with biliary flow [2544].

Suppurative Cholangitis. The causes and consequences of suppurative cholangitis differ little from those of the intrahepatic form. The adnexal glands of the ducts are not a common source of infection. The lesion is rare in *Escherichia coli* infections but more common in streptococcal infections [281].

Cicatrizing Cholangitis, or Strictures. Calculi in the extrahepatic bile ducts produce decubital ulcers (Fig. 104, upper left), which usually heal, leaving a small insignificant scar. Occasionally a stricture results (Fig. 104, middle). Benign strictures in the biliary tracts (Fig. 104, upper right) frequently result from trauma to the mucosa of the extrahepatic ducts, resulting in secondary inflammation and scarring [1432, 3476, 3477]. The most common cause of the trauma is surgical intervention during which the duct is clamped, tied, incised, or lacerated [518], especially if it is poorly exposed during surgery. The least common cause is a primary inflammation, which may heal with scarring of the wall, with or without obstruction of the lumen [687, 1900]. A similar lesion may also be produced by a cellulitis in the gastrohepatic omentum following cholecystectomy [1244].

The treatment of strictures is reparative surgery [3476]. Spontaneous rupture of inflamed ducts, which usually contain stones, may occur, resulting in biliary peritonitis [574, 2374].

MORPHOLOGY OF FATTY METAMORPHOSIS

Distribution of Fat in the Hepatic Cell. In the normal liver, fat is found in the hepatic cells and in the Kupffer cells in the form of small droplets (Fig. 6, lower left), the amount demonstrable depending on the sensitivity of the histologic method used. In contrast, in the rat the demonstration of any fat with routine methods points to an aberration from the physiologic normal. Under various abnormal circumstances, the amount of stainable fat rises; it is associated with a sharp increase of the chemically extractable lipids. Transitions between the normal and the unequivocally abnormal states exist, the pathologic significance of which is not easily determined. Various patterns of abnormal histologic distribution of fat exist. They have been classified according to the etiologic factors causing the fat increase [2833, 3433].

PERISINUSOIDAL PATTERN. The physiologic distribution of fine droplets, like beads on a string, just beneath the sinusoidal surface of the human hepatic-cell plates becomes exaggerated under abnormal circumstances, so that fat droplets of 1 to 2 μ in diameter line the sinusoidal surface, not interrupted by the cell borders. The fat droplets are not shells around mitochondria, although the mitochondria are altered under such circumstances [752].

PERINUCLEAR OR PERIBILIARY PATTERN. In the center of the hepatic cells, fine fat droplets, especially numerous in the presence of much lipofuscin pigment, are like shells around the pigment. These droplets reflect the distribution of lipofuscin, rather than alterations in fat metabolism. Older lipofuscin does not give a fat reaction [2863].

DIFFUSE SMALL-DROPLET PATTERN. Perisinusoidal distribution may progress so that fat droplets of small to medium size—up to 4 μ —are irregularly distributed throughout the cytoplasm of the hepatic cell. Eventually the cytoplasm of the hepatic cell is almost completely displaced by these fat droplets. They do not displace the nucleus, but give a foamy appearance to the cytoplasm in routine paraffin sections. This is not necessarily impressive, and a chemically demonstrable increase in fat content (up to 15 per cent of the wet weight) may be associated with barely recognizable histologic changes. For this reason paraffin sections are unreliable in estimating the degree of fatty metamorphosis.

LARGE-DROPLET DEPOSITION. The previous stage may proceed further, to coalescence of the small droplets to large droplets, so that one or two fat droplets up to 10 μ in diameter are present in the hepatic cells, in addition to many fine droplets. Finally one large drop replaces almost the entire cytoplasm of the hepatic cell and pushes the nucleus to one side. The nuclei are pyknotic and without a nucleolus. This suggests reduced metabolic activity of such cells. The large drop measures up to 20 μ in diameter. The small cytoplasmic rim under these circumstances may contain a considerable amount of basophilic material and glycogen [1682]. Glycogen is also stained within the fat droplet, although this may be an artefact [2652]. The large fat droplets may contain a protein film. Their vitamin A fluorescence is usually lower than that of the finer droplets [2625].

"FATTY CYSTS." In fatty metamorphosis of longer standing, large fatty cysts, up to 100 μ in diameter (thus considerably exceeding the size of individual cells), are noted (Fig. 105B). Cysts are conspicuous in rats or mice kept on choline-deficient diets

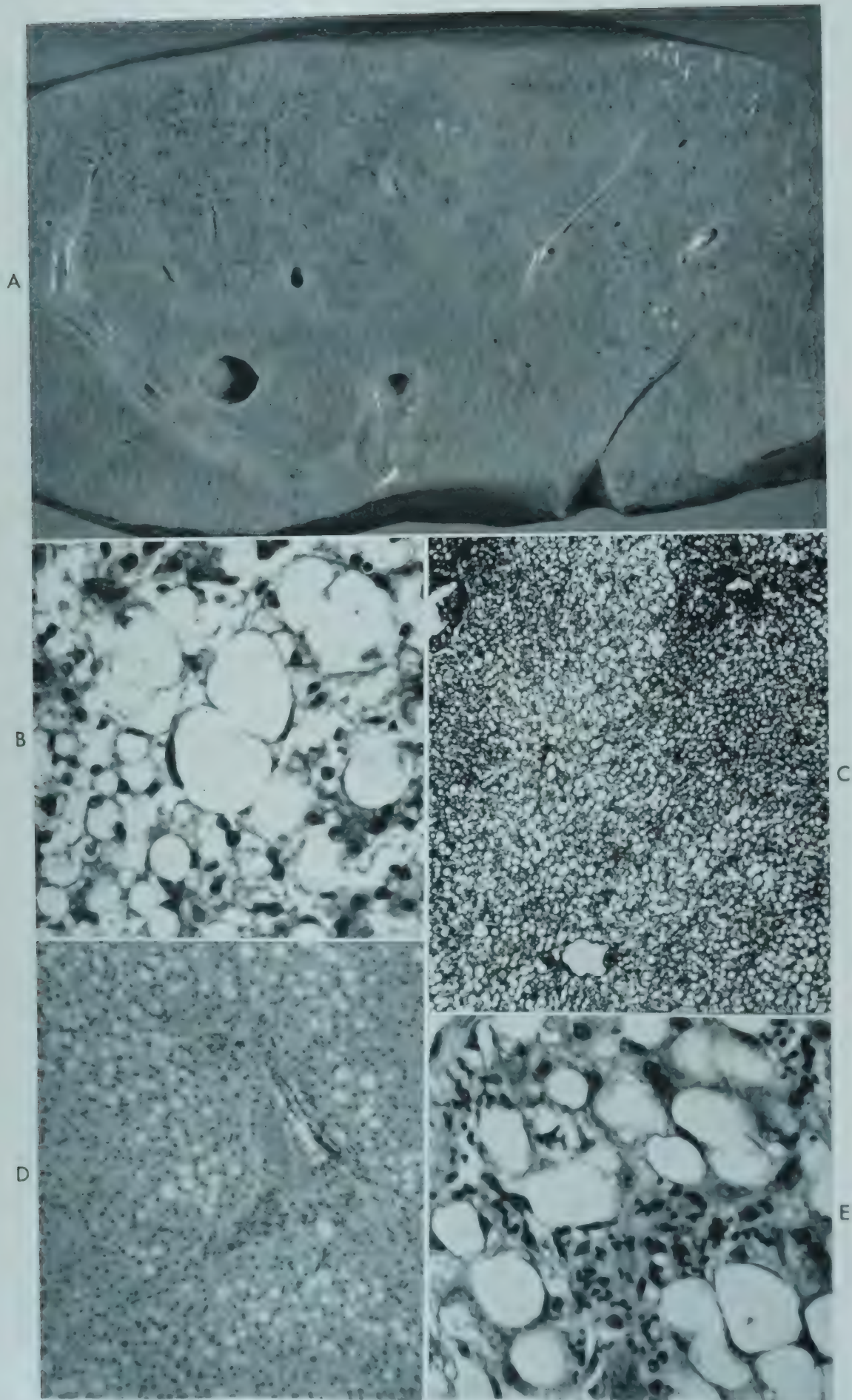


FIG. 105 A. Gross picture of fatty liver, with central necrosis exaggerating the lobular markings in the left upper corner. B. Fatty cysts resulting from coalescence of fat

for a prolonged period [1405]. These cysts are not dilated individual cells but have formed by coalescence of large fat globules in individual cells. The adjacent portions of the cell membranes between the fat globules first become considerably stretched and subsequently rupture and disappear. The final product is a multicellular structure lined by the dedifferentiated cytoplasmic portion of the hepatic cells. Many nuclei—up to 80—are found on their periphery. The development of these fatty cysts permits the storage of much more fat within the liver than would be possible if the fat remained in individual cells.

Kupffer Cell Fat. Excessive fat in Kupffer cells is found in diabetes [2797], sometimes in contrast to the absence of fat in the neighboring hepatic cells. A similar discrepancy, although with less fat, is found in cancer [3433]. On the other hand in fatty liver, particularly if associated with cirrhosis, the Kupffer cells may contain relatively little fat, in comparison with the hepatic cells [1172]. If sufficiently sensitive methods are used, the Kupffer cells in man are never found to be free of fat [3433], while in animals, e.g., vitamin A-deficient rats, the reverse may be true. In general, Kupffer cell fat parallels that of the hepatic cells.

Fat in Portal Tracts. In some forms of cirrhosis or hepatitis, in chronic passive congestion, and in the fatty liver, small amounts of fat are found in the portal tracts, either in macrophages or free in the tissue. In septal cirrhosis small amounts of fat may also be found in the septums in the form of fatty cysts [1484]. Fatty cysts in the portal tracts in the absence of fatty metamorphosis indicate a previously existing severe fatty metamorphosis.

Lobular Fat Distribution. The distribution in the lobule is independent of the type of fat distribution in the cell, except that perinuclear fat usually is centrolobular. Nutrition and the interplay between deposition and removal of fat in the liver owing to nutritional factors probably have a role in localizing the fat.

SCATTERED FAT. Isolated fat-rich hepatic cells are found throughout the lobule in otherwise apparently normal livers. Even small scattered groups

of such cells are not abnormal in man. This is seen after a diet extremely high in carbohydrate. Scattered large cysts may be the result of therapy in a previously fatty liver, the large droplets having not yet responded [1682].

CENTROLOBULAR FAT. Fat in the center of the lobule occurs typically in anemic or hypoxemic conditions; eventually the greater part of the lobule is involved. The hepatic cells sometimes become necrotic or disappear. Central accumulation of fat is also induced by nutritional factors without hypoxemia, as in alcoholic persons (Fig. 105C) or in choline deficiency [1405]. The fat appears first and disappears last from the center. Central fat distribution is recognized grossly, the yellow centers of the lobule standing out from the brown, usually anemic peripheral zone.

INTERMEDIARY FAT. Fat is found in the intermediary zone mainly if the central zone has been destroyed or damaged and the intermediary zone is the most central intact area. It is found in severe passive congestion, toxic central necrosis, and, typically, in experimental carbon tetrachloride intoxication.

PERIPHERAL FAT. Predominantly peripheral accumulation of fat is found in toxemias caused by many diseases in which the liver is not the primary target. It is also sometimes noted in nutritional fatty livers (Fig. 105D). In some forms of experimental malnutrition, fat predominantly accumulates in the peripheral zone. This also occurs in protein deficiency in the presence of adequate amounts of choline. It appears on the periphery as a result of mobilization from the depots after administration of anterior pituitary preparations to starving rats.

DIFFUSE FAT. Diffuse fatty metamorphosis (steatosis or lipidosis) presents an impressive gross picture of an enlarged and yellow liver with a doughy consistency and a greasy appearance on the cut surface. The lobular markings are almost completely obscured (Fig. 105A). Histologically, nearly every cell contains excess fat. In some instances the fat forms small droplets, producing foamy cells. In others it forms individual large drops in each cell. Both distributions may be

droplets in neighboring hepatic cells in fatty liver in an alcoholic. H&E ($\times 230$). (Popper, H.: *Am.J.Med.* 16:98, 1954.) C. Diffuse fatty infiltration with central predominance in fatty liver in an alcoholic. Mallory's aniline blue ($\times 60$). D. Peripheral fat distribution in fatty liver in an alcoholic. H&E ($\times 90$). E. Focal necrosis with segmented leukocytes replacing necrotic hepatic cells in fatty liver in an alcoholic. H&E ($\times 330$).

found with almost identical chemical fat values of up to 20 per cent of wet tissue. Originally, the small droplet form was supposed to represent degeneration, while the large drop form was termed infiltration. This differentiation can no longer be maintained; the differences are the result of variations in duration and speed of fat accumulation. In fatty livers with 20 to 40 gm fat per 100 gm tissue, large droplets predominate. In diffuse fatty metamorphosis, the sinusoids are narrow and usually free of red cells. The tissue spaces are not expanded even in necropsy specimens, suggesting that the fat-laden hepatic cells exert pressure on the sinusoids and spaces. Since the diffuse fatty liver is an exaggeration of the central and peripheral forms, it is usually nutritional, toxic, or endocrine in origin.

PATCHY FAT DISTRIBUTION. In a variety of conditions, including sudden accidental death and liver diseases, sharply limited foci are seen consisting of parenchymal and Kupffer cells rich in fat, with the surrounding parenchyma uninvolved. These foci have no relation to the lobular architecture. They are probably a result of agonal processes related to the blood flow rather than of intravital functional differences of these zones.

Appearing and Disappearing Fat. The development of the choline-deficient fatty liver is taken as an example of developing and disappearing fatty metamorphosis, although the same pattern is seen in many other experimental lesions. The small fat droplets on the edge of the hepatic cells become larger, primarily in the center of the lobule. The perisinusoidal pattern changes to diffuse fatty metamorphosis, and subsequently large fat drops and fat cysts form, depending upon the duration of choline deficiency. If choline is restored to the diet, the fat removal follows the reverse of the process of development. Fat supposedly leaves fatty cysts through the adjacent bile canaliculi or sinusoids even while the deficiency persists, because the walls of these vessels have become partially opened during the development of these cysts. During repletion, fat reenters the cytoplasm around the cyst in the form of fine droplets, so that a greater part of the fat gradually moves from an extracellular to an intracellular location and thus can be utilized by the liver. Gradually, the fat cyst disappears, with subsequent restoration of the architecture. Rosette-shaped arrangements of the hepatic cells around cysts are considered morphologic evidence of regression of fatty metamorphosis [1408]. In contrast, the emptying of a cyst

during persistent deficiency leads to its collapse, with approximation of the surrounding framework (see Structural Changes, under Hepatic Injury from Dietary Imbalance or Multiple Factors, Chap. 50).

Relation of Chemical to Histologic Fat. The content of total lipid in the normal human liver varies from 2.4 to 8.4 gm per 100 gm wet weight, while more than 9.0 gm per 100 gm is found in fatty livers parallel with an elevation of the serum-cholesterol level above 300 mg per 100 ml [2703]. Chemical differences have been claimed to exist between the normal and abnormal liver fat [3319], but this claim has not been substantiated [2572].

Chemical analysis coincides fairly well with the amount of fat seen histologically if specific stains, particularly the sensitive ones, are used. The appearance in paraffin sections is misleading. In the earlier literature, the concept of fat phanerosis was emphasized, implying histologically visible fat without a corresponding increase in chemical fat. This was said to be caused by changes in the physicochemical nature of the dispersed fat. Such a possibility can not be denied, but it plays a minor role in the development of the fatty liver.

ETIOLOGY OF FATTY METAMORPHOSIS

Basic Factors. Fatty metamorphosis is an imbalance of the normal processes governing the fat content of the liver and not the result of an abnormal metabolic pathway. In this sense fatty degeneration, implying transformation of hepatic-cell cytoplasm into fat, does not exist [3212], with the possible exception of fat phanerosis. In principle (see Fig. 15), the excess fat is the result of (1) increased formation of fat; (2) decreased oxidation of fat; (3) increased flow of fat from the depots to the liver; or (4) impaired removal from the liver. Increased metabolic or growth stimuli can overtax a relatively deficient removal mechanism, resulting in fatty liver. In the removal mechanism, deficiency of the lipotropic agents, especially choline and methionine, is important (see Methionine Deficiency, under Nutritional Deficiencies, and Lipotropic Factors, under Lipogenic-Lipotropic Imbalance, Chap. 50). The demand for choline is exaggerated by other factors which tend to cause fatty metamorphosis, such as a low-protein-high-fat diet. Protein is probably required for structural enzymes necessary in fat metabolism [787]. Some enzymes concerned with lipid metabolism are probably located in the mitochondria

[1726]. Reduction of enzymes as a result of protein deficiency is well established [1820, 2382]. Of less importance is a deficiency of unsaturated fatty acids necessary in the formation of the phospholipid molecule [2572].

Mechanism of Fatty Metamorphosis. The pathogenetic mechanisms for many causes of fatty liver are poorly understood. In principle, three mechanisms can be distinguished. The first is imbalance of nutritional, endocrine, or neurovegetative factors in which an excess or deficiency of one factor can be corrected by an excess or deficiency of another. For instance, the fatty liver produced by a high-fat diet is corrected by high-protein intake. Starvation or slowing of growth with maturation may make the demands for deficient factors, such as choline, less urgent and thus "correct" such a fatty liver. In this type of fatty liver, therefore, an interplay of antagonistic and synergistic factors exists which makes the understanding of the etiology in a given case extremely difficult. The fatty liver in central nervous system disorders such as mongolism or gargoylism probably belongs to this group.

The second type of mechanism is induced by toxic or bacterial factors which apparently interfere with the enzymes concerned with fat metabolism. It is less dependent upon nutritional or endocrine factors and is not completely counteracted by them. The third type of mechanism is the result of anoxia.

Interplay of Mechanisms. The division into these three mechanisms is not sharp. For example, some toxic substances, particularly chemicals, produce deficiency. Guanidoacetic acid and niacinamide (see Vitamin B Complex, Chap. 8) produce fatty liver because of diversion of methyl groups by binding them. This is an example of detoxification taking precedence over other metabolic functions. The beneficial effect of methionine in chloroform or carbon tetrachloride intoxication in dogs could be interpreted as correction of a conditioned methionine deficiency [2299]. The fatty liver found in ulcerative colitis or intestinal tuberculosis [1656] in man may be caused by absorption of toxic substances from the intestine, nutritional deficiencies, or anemia. In the alcoholic fatty liver, toxic factors play a role, although imbalance is the major pathogenetic mechanism. The main justification for an etiologic subdivision of fatty liver is the persistence of nutritional or hormonal imbalance resulting in the clinical problem of fatty liver, while the fatty metamorphosis in most examples of

toxic or anoxic fatty liver is usually mild and transient. If it becomes severe, as when it is the result of a poison, it is overshadowed by necrotic changes in the liver and does not present the problem of clinical fatty liver.

Examples of Etiologic Factors of Fatty Metamorphosis of the Liver

I. Imbalance

A. Nutritional factors

1. Starvation
2. Low-protein diet
 - a. Methionine deficiency
 - b. Cystine deficiency
3. High-fat diet
4. High-carbohydrate diet
5. Lipotropic deficiency
6. Vitamin imbalance
 - a. Thiamine excess
 - b. Biotin excess

B. Metabolic factors

1. Pituitary hormones (ACTH, adipokinin)
2. Adrenal cortical hormones
3. Thyroid deficiency or excess
4. Insulin deficiency (diabetes mellitus)
5. Sex hormones
6. Central nervous system influence
7. Obesity

II. Toxic factors

A. Chemical poisons

1. Carbon tetrachloride
2. Chloroform
3. Phosphorus
4. Trinitrotoluene

B. Bacterial toxins

C. Anoxic factors

1. Anemia
2. Congestion

III. Combined factors

A. Alcoholic fatty liver

FUNCTIONAL MANIFESTATIONS

The functional manifestations of fatty liver are not easily demonstrated, because accompanying hepatocellular degeneration or fibrosis can not be excluded in most clinical conditions.

Experimental Observations. Acute fatty liver produced by ethionine administration to female rats is associated with Bromsulphalein retention and increased serum bilirubin, while the serum and hepatic enzymes are unchanged [1820]. The same findings are obtained in the fatty liver produced by 4 weeks' administration of a choline-deficient diet. The fatty livers produced by a high-fat-low-protein diet or by carbon tetrachloride are

associated with hepatic-cell damage, and enzyme changes are noted [1821].

Some not fully established evidence exists of impairment of sinusoidal circulation by fat-laden cells [1497]. Bromsulphalein retention and hyperbilirubinemia in the pure experimental fatty liver are therefore possibly an expression of impaired circulation rather than of impaired function. The reported increase of phosphatase activity in serum [258, 261, 2142] may be an expression of associated hepatic injury. Elevation of pseudocholinesterase activity in the plasma of male rats on choline-deficient diets is thus far not explained [1433]. Reduction of the basophilia of the hepatic cells in the presence of acute fatty liver suggests impaired protein formation [976]. Regeneration is not retarded in the fatty liver. In fatty livers produced by choline deficiency, the biliary secretion is reduced and the lipid level of the bile is lower than normal [631]. Other functions, such as growth and reproduction, are not impaired in the presence of fatty liver [1610]. Abnormalities in the glucose tolerance have been explained by a disturbance of the hepatic hexokinase system in fatty metamorphosis. Moreover, in fatty livers produced experimentally by low-choline and -methionine diets, the hepatic glycogen content is increased [953] and glycogenesis from substances other than glucose may be intact. The excretion of coproporphyrin III in fatty liver is probably not related to the fat but to other concomitant alterations [3508]. Whether detoxification is impaired in the uncomplicated acute fatty liver is not fully established. Therefore, no convincing indication is available of significant impairment of hepatic function by experimental fatty metamorphosis, despite the apparent reduction of hepatocellular cytoplasm.

Observations in Man. If the liver biopsy specimen reveals no other alterations, increased Brom-

sulphalein retention is the only fairly consistent abnormal result of the hepatic tests in fatty metamorphosis [431, 1075, 3386]. Abnormal glucose tolerance is also associated with fatty metamorphosis in man [3043] and has been related to "hepatic" diabetes.

SEQUELAE

The fact that the acute fatty liver entails surprisingly few functional alterations does not imply that the presence of fat is harmless.

ACUTE NECROSIS. In the human fatty liver, necrotic foci are frequently encountered, either focally or centrally (Fig. 105E). Moreover, patients with fatty liver, particularly on a nutritional basis, succumb rapidly to hepatic insufficiency (see The Nutritional Fatty Liver-cirrhosis Syndrome, Chap. 51). The reasons for this increased susceptibility to injury of the fatty liver are not clear. The alleged disturbance of the sinusoidal blood flow could facilitate anoxia and necrosis. Detoxification of injurious substances, especially of bacterial origin, may be faulty, and the fatty liver may be more readily sensitized to hepatotoxins. Some of the hepatotoxins are soluble in the fat [3212]. Sudden death may occur in young adults with fatty liver [1245, 1927].

FIBROSIS AND CIRRHOSIS. Prolonged fatty metamorphosis, either clinical or experimental, often leads to fibrosis and cirrhosis formation in the liver [319, 530, 1320, 2002]. Some investigators have considered fatty metamorphosis itself the cause of cirrhosis [640, 1407, 1497]; others have denied this [739, 783, 1318]. The pathways of fibrosis and cirrhosis formation will be discussed in detail in the next two chapters. Hepatoma formation may be the end stage of the hepatic injury due to fatty liver, as illustrated in the choline-deficient rat [2903].

An apparent or actual increase of the connective tissue of the liver may occur locally around a circumscribed lesion, such as an abscess or tumor, or may involve almost every lobule. The term "fibrosis" is applied even if membranes and not fibers are responsible for the increase in connective tissue [911]. In the microscope sections, fibers usually appear as commas or dots, depending on the plane of sectioning, while membranes that are two-dimensional sheets appear as straight or wavy lines [911]. Fine membranes which appear as wavy lines in sections may aggregate to form thicker membranes and eventually septums. As two-dimensional sheets also, the septums traverse the lobules and appear as bands [2630]. In principle, three different processes cause fibrosis:

1. Alteration of preexisting fibers or membranes
2. Approximation of preformed fibers or membranes, after necrosis, and disappearance of intervening hepatic cells, or collapse
3. New formation of fibers or membranes, either by fibroblasts or without them

The differentiation of the three mechanisms in the individual case is difficult, as the preexisting fibers may be altered in collapsed areas, or new fibers or membranes may be formed in addition to approximation of preformed fibers.

This poses the question whether hepatocellular degeneration and necrosis cause fibrosis, aside from that which results from collapse. Some investigators consider parenchymal injury and fibrosis as parallel and not causally related processes [1172]. Others assume a local stimulating effect similar to any other chronic inflammation [116, 1696, 2337, 2359]. "Collagenization" of preformed fibers occurring typically in the vicinity of degenerating hepatic cells [116] may reflect al-

teration of fibers, rather than new formation. In addition, intralobular parenchymal injury is frequently associated with portal inflammation and subsequent periportal radiating membrane formation. This is a result of drainage of cellular breakdown products to the portal areas [467, 1497].

Fibrosis without cirrhosis in any of its forms has little functional significance [1696]. Even severe and diffuse involvement does not disturb hepatocellular function, hepatic circulation, or bile flow [3183].

The attempt to differentiate these forms of fibrosis is useful in diagnosis and in the study of morphogenesis. Collapse as well as new formation is best characterized by lobular distribution and extent.

FORMS OF FIBROSIS

Changes of Preformed Fibers

Intralobular as well as portal fibers may be altered. Within the lobule, where collagen membranes are normally sparse, reticulum fibers may become coarse and take the staining reaction of connective tissue with Van Gieson stain. This occurs in a patchy arrangement with increasing age. It is the result of metabolic changes, as in diabetes [2797], or of local stress in passive congestion or around areas of regeneration. Actual transformation of reticulum fibers into collagen membranes is not observed, although strongly suggested [849]. The reticulum fibers may serve only as a framework [2630]. Coarsening of the fibers in the portal tracts is also an aging process which may be only focal, and in arteriosclerosis and hypertension such fibrosis occurs without functional changes. A circular type of fibrosis

develops in prolonged extrahepatic cholestasis. This eventually involves collagen fibers around the intralobular trabeculae containing ductules and arterioles.

Fibrosis Following Collapse

If hepatic cells disappear, the denuded framework collapses. As a rule, regenerating hepatic cells extend quickly into the denuded area and the architecture is not significantly changed after regeneration if the connective tissue framework is intact [2187]. For instance, after fairly extensive necrosis in viral hepatitis, the replacement of the hepatic cells is so complete that fiber stains fail to show any abnormality after recovery [2083]. However, in conditions in which regeneration is prevented, the collapse is permanent. This happens (1) when residual breakdown products or bile imbibition remain and inhibit reexpansion; (2) when an entire lobule is wiped out and no hepatic cells are left to regenerate; (3) when new formation of fibers in the collapsed areas distorts the pattern; (4) when repeated attacks follow each other [467]; (5) when the connective tissue fibers or membranes break, as in some forms of toxic hepatitis [2625]. This last-named phenomenon is associated with new formation of fibers.

Collapse of Entire Lobules Following Massive Necrosis. If all hepatic cells of an entire lobule are wiped out by an acute necrotizing process and the subsequent exudate disappears, the framework collapses (Fig. 106D). Between the accumulated connective tissue membranes, a variable sprinkling of exudate cells and regenerating ductules is noted. Often several lobules are involved in massive necrosis. This results in wide, grossly visible bands of connective tissue, in which the regular spacing of the approximated central and portal fields indicates the pathogenesis of the process (Fig. 106A). If previously normal liver parenchyma has undergone massive necrosis, or primary collapse, the approximated portal and central fields retain their original relationship to one another and the demarcation of the shrunken lobules—ghost lobules or “no man’s land”—is readily discernible (Fig. 106B). In addition to the preformed approximated reticulum fibers, membranes visualized with Mallory’s aniline blue stain traverse the ghost lobules. These differ from the thick fibers of the original portal tracts and central fields, so that the borders of the portal tracts and central fields are apparent with connective tissue

stains (Fig. 107B) [2630]. Most sinusoids in the ghost lobules are also collapsed. The few remaining open are short shunts between branches of the portal vein and hepatic artery on one side and hepatic vein branches on the other.

Massive collapse may involve cirrhotic parenchyma, i.e., secondary collapse. In these instances, the pattern of the portal and central fields is irregular, and the demarcation of the portal tracts from the lobular parenchyma becomes hazy. Sudden massive necrosis of several lobules produces planes of stress or stress fissures in the surrounding intact parenchyma, along which hepatic cells disappear [2631]. These stress fissures do not follow the lobular topography (Fig. 107C). The effect of these fissures, as well as the portohepatic shunts, is discussed under cirrhosis.

Submassive Collapse. Since submassive necrosis is more common than massive necrosis, partial or submassive collapse of lobules is also more frequent. The residual lobular fragments may vary from small parts of one lobule to large areas containing several lobules (Fig. 107D). The distortion of the collapsed framework by these fragments interferes with reexpansion, especially if regeneration occurs irregularly and extensively in them (Fig. 106C). This regeneration, which leads to nodule formation as well as further dissection by break fissures (Fig. 108B), belongs to the complex of cirrhosis.

Submassive Collapse Following Repeated Episodes of Focal Necrosis. Repeated episodes with many areas of focal necrosis may reduce the parenchyma to a degree equal to that following submassive necrosis. This condition has been experimentally produced by prolonged ethionine feeding, and apparently occurs in protracted human viral hepatitis.

Central Collapse. If hepatic cell necrosis involves only the central area, central collapse results if regeneration does not quickly follow (Fig. 91C). Experimentally, this develops after repeated exposures to carbon tetrachloride [467] or bromobenzene. In man, it frequently results from chronic passive congestion. Central collapse is frequently associated with new formation of membranes and is thus not purely a collapse phenomenon. Connection of central areas of collapse by bridges represents a transition to submassive collapse.

Periportal Collapse. Periportal necrosis, portal inflammation, and, less frequently, bile infarcts lead to periportal collapse. However, new forma-

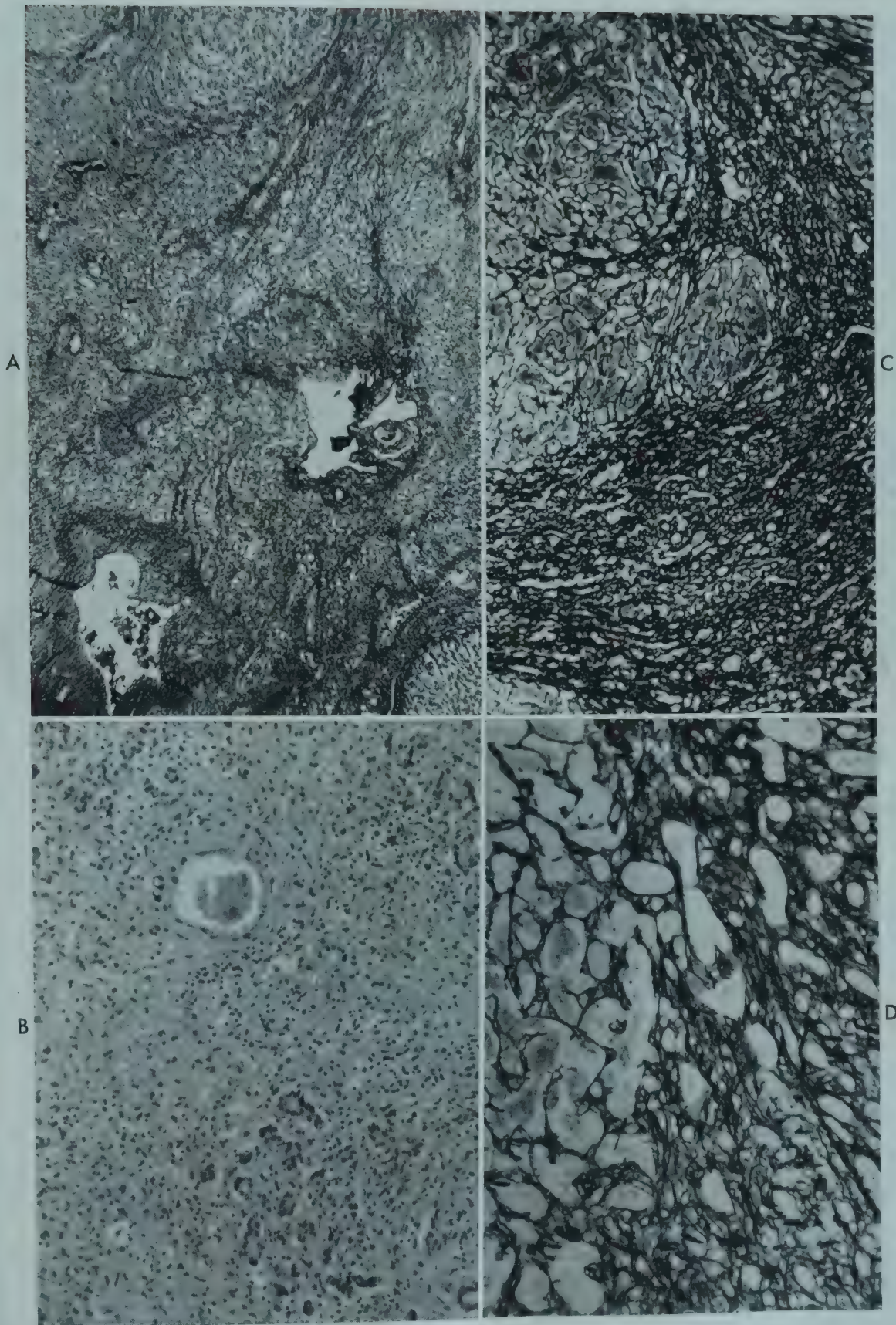


FIG. 106 A. Massive collapse of framework with approximation of central and portal canals. The structure of the portal canal differs from that of the collapsed framework. Mallory's aniline blue ($\times 52$). (Popper, H.: *Am.J.Med.* 16:98, 1954.) B. "Ghost" lobule after collapse of the framework in viral hepatitis. The periphery of the lobule is demarcated by proliferating ductules. H&E ($\times 105$). C. Collapse of framework around persisting liver tissue. Gomori silver impregnation ($\times 52$). D. Detail of collapse of framework in viral hepatitis. Gomori silver impregnation ($\times 120$).

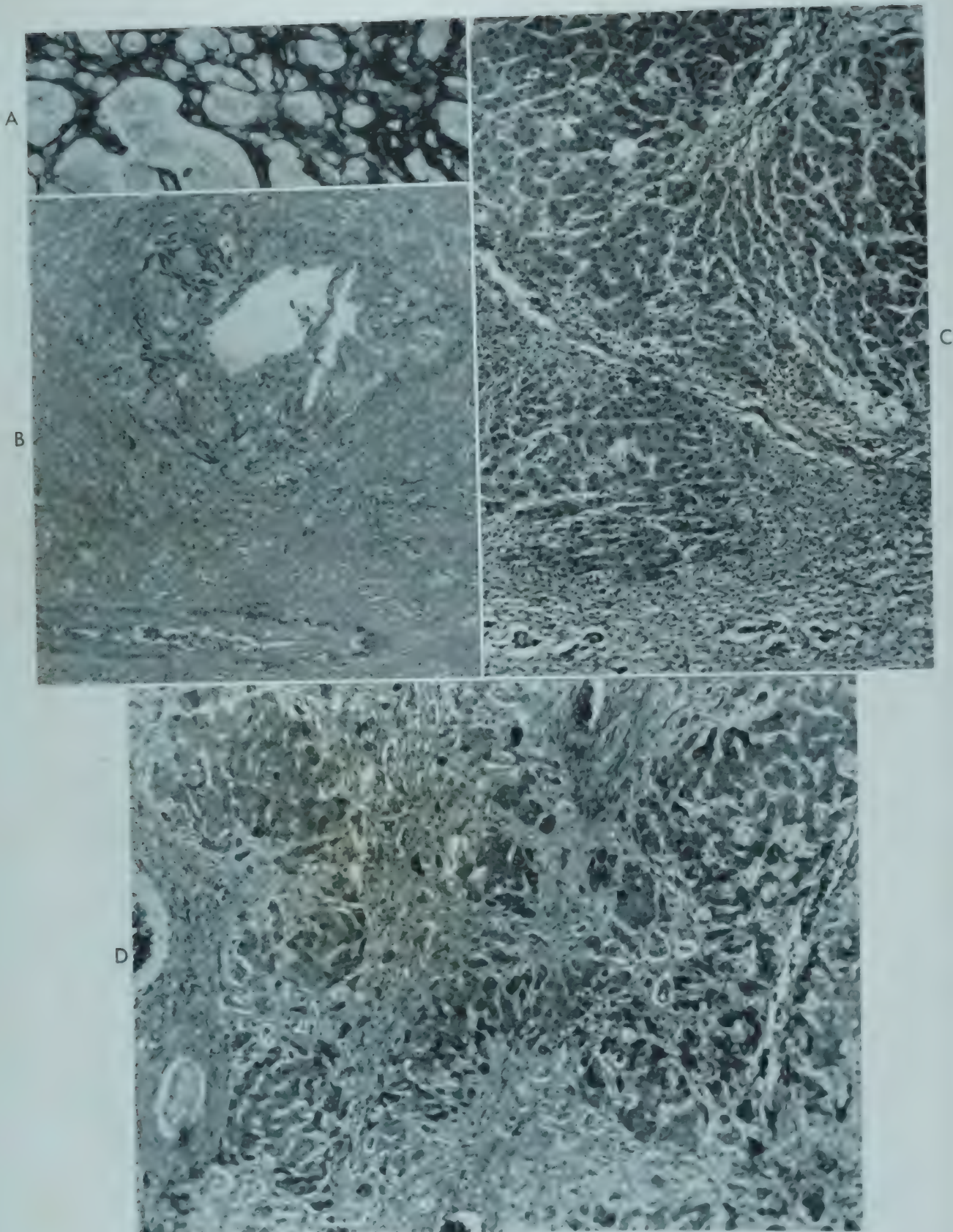


FIG. 107 A. Intralobular collapse of framework after disappearance of hepatic cells. Gomori silver impregnation ($\times 265$). B. Detail of approximation of portal and central canal in postnecrotic massive collapse. Note hazily stained membranes in the collapsed area, in contrast to the thick collagen fibers in the portal and central canal. Mallory's aniline blue ($\times 12$). C. Fissures in intact lobular parenchyma bordering on an area of recent postnecrotic collapse. In region of fissure, note loss of hepatic cells about the dilated sinusoids. H&E ($\times 65$). (Pepper, H., Elias, H., and Petty, D.: *Am.J.Clin.Path.* 22:717, 1952; courtesy of The Williams & Wilkins Company.) D. Submassive collapse in subacute viral hepatitis. H&E ($\times 65$).

tion of periportal membranes is more important (see Periportal Membrane Formation, later in this chapter).

Focal Intralobular Collapse. If hepatic cells disappear in focal necrosis, the framework collapses temporarily. If reexpansion of the framework is prevented by one of the processes mentioned above, focal intralobular scars result (Fig. 107A). New formation of fibers in such lesions is a common occurrence. The reticulum framework around fatty cysts is transformed into collagenous membranes, probably stimulated by mechanical pressure from the developing cyst. When the cyst ruptures and discharges its fat content into bile canaliculi or sinusoids, the membranes become approximated and a fibrous scar forms [1407] (Fig. 111B).

Fibrosis from New Formation of Fibers or Membranes

New formation of fibers or membranes occurs with or without fibroblasts. Fibroblasts are seldom recognized within the lobular parenchyma, and therefore the formation of fibers without fibroblasts has been assumed and has been thought to be chiefly stimulated by protein which has escaped into the tissue spaces [2797]. In "serous hepatitis" [945], increased sinusoidal permeability for protein was considered responsible for the extracellular fiber formation. Such fiber formation in turn has been held to be detrimental to the surrounding hepatic cells. Other examples of acellular fiber formation were cited in analogy. Not only serum protein, but macromolecular components of disintegrated hepatic cells may serve as a frame along which fibers develop. This would be an analogy to the transformation of collagen in solution which assumes the electron microscopic characteristic of periodicity of fibers if exposed to macromolecular substances acting as a frame. Recently fiber formation in the tissue spaces, "collagenosis," allegedly caused by nutritional disturbance, has been designated as "serous hepatitis" [1491]. New formation of fibers can not easily be differentiated from the previously mentioned transformation of reticulum fibers into collagen membranes. Moreover, the frequently seen hepatic edema, "serous hepatitis," is mostly agonal and usually pronounced in the central zone, while fibrosis is more prominent in the peripheral zone. Therefore fibers formed in the tissue spaces on the basis of edema or hepatocellular breakdown products require further histo-

chemical investigation. Around inflammation or irritation in the lobular center or periphery or within the lobular parenchyma, fibers or membranes may form near cellular exudate which may be transformed into fibroblasts.

Intralobular Formation of Membranes or Fibers. In the lobular parenchyma, fibrosis occurs around focal degeneration and necrosis from any cause and is exaggerated in and around granulomas such as tuberculous, typhoid, brucella, or sarcoid nodules. Not only does a scar persist, but membranes radiate from the necrotic or granulomatous focus into the parenchyma (Figs. 108A, 112A). Fibers or membranes also develop in intraparenchymal fissures resulting from stress. For example, such fissures radiate from areas of massive collapse as a result of the centripetal stress exerted by the collapsed area. Another type of stress fissure develops on the border of territories of hepatic parenchyma exhibiting unequal tissue turgor. This may occur between two areas with (1) unequal fat content; (2) unequal regeneration; (3) unequal expansion because of tumor or abscess formation; (4) unequal collapse following necrosis (Figs. 107D, 108B). In the fissures, hepatic cells disappear and the framework collapses. A sheetlike scar, seen as a straight line in histologic sections, develops in which some dilated sinusoids persist. Collagen membranes are deposited, and the sinusoids become transformed into venules [2630, 2631]. The membrane formation is aggravated by the pressure exerted by regeneration on one or both sides of the fissure. Eventually a straight septum develops traversing the lobular parenchyma, often starting from the portal tracts (Fig. 112D).

Central Membrane Formation. Thin membranes radiate from the central field in the tissue spaces of the surrounding parenchyma (Fig. 108C). This results from congestion, bile stasis, or disturbed intralobular blood flow caused by portal vein thrombosis or an Eck fistula, for instance. Disturbance of sinusoidal blood flow by excessive fat or glycogen deposition has been accused of initiating the same process [640, 1173, 1497]. Radiating membrane formation is conspicuous if associated with central necrosis on a congestive or toxic basis. The subsequent collapse accentuates the central fibrosis, resulting in thicker membranes and eventual septum formation. Some of the septums formed from radiating central membranes connect neighboring central fields, especially in passive congestion. Membranes also radiate from

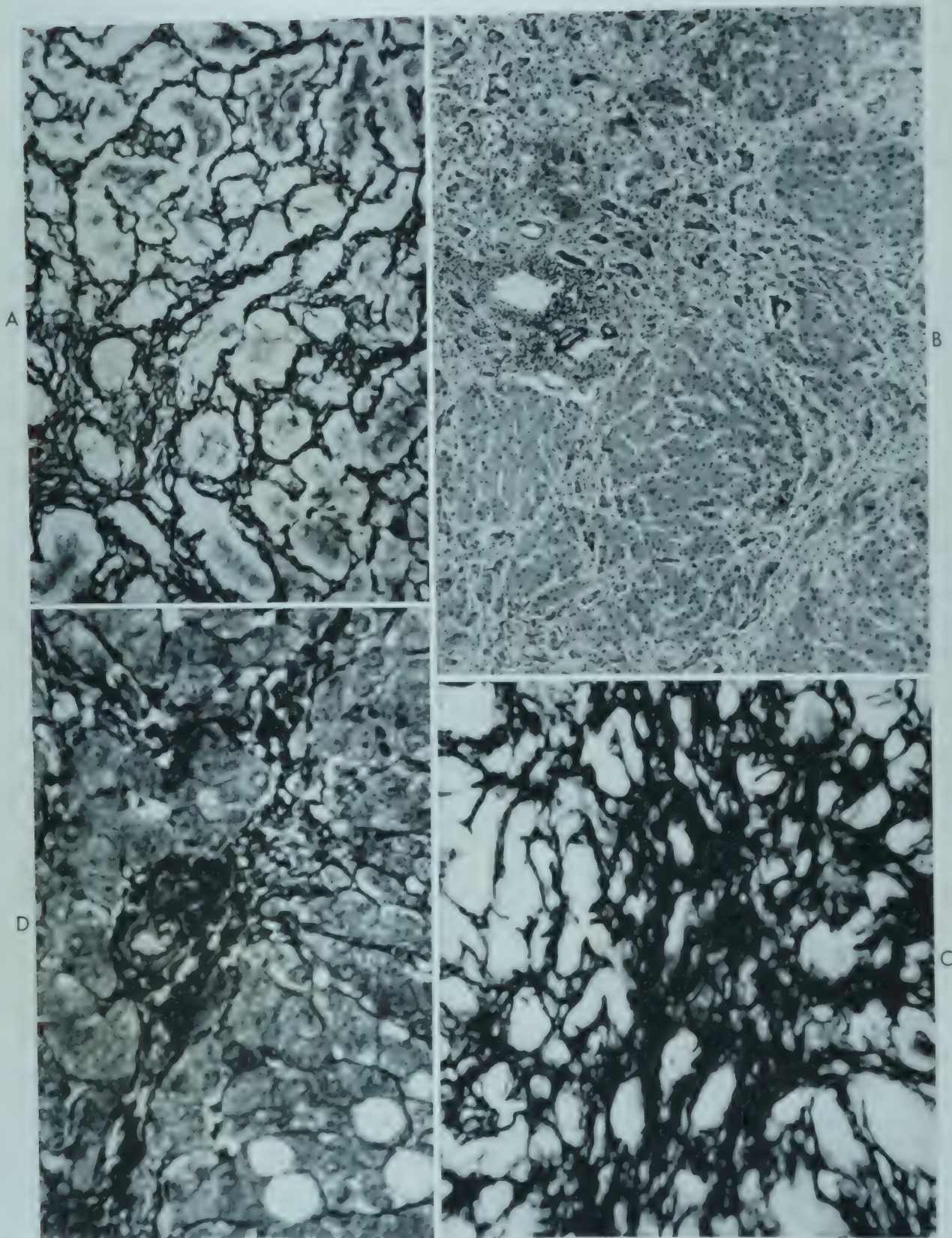


FIG. 105. A. Increase in intralobular reticulum fibers near area of necrosis. Gomori silver impregnation ($\times 115$). B. Stress fissures around regenerating nodules in submassive necrosis. H&E ($\times 52$). C. Central membrane formation with disappearance of some hepatic cells in chronic passive congestion. Gomori silver impregnation ($\times 220$). D. Peripheral membrane formation starting from portal tract in periportal inflammation. Mallory's aniline blue ($\times 250$).

the central field around collapsing fatty cysts (Fig. 111B).

Periportal Membrane Formation. Portal inflammation stimulates the formation of membranes radiating from the portal tracts into the tissue spaces (Fig. 108D). The portal fields appear stellate in sections (Fig. 111C) [739]. This results from nonspecific or specific (viral or granulomatous) inflammation or from chemical irritation by hepatocellular breakdown products, bile imbibition, iron in hemochromatosis, or quartz deposition following its experimental intravenous injection [1398]. Periportal fibrosis becomes more prominent if associated with periportal necrosis and destruction of the limiting plate. Collapse and new formation of membranes produce thick membranes, which eventually condense to form septums. These septums extending in the forks of the portal vein branches finally link neighboring portal tracts. Perilobular fibrosis develops, with anatomic demarcation of the lobules from each other (Fig. 112B). With arrest of the inflammatory process and subsequent regeneration, the perilobular septums become compressed into thin sheets, which may completely disappear, as observed in serial liver biopsy specimens [215]. Fibroplasia via vascular granulation tissue has been considered important in septal cirrhosis [2359].

Periductular Fibrosis. In prolonged extrahepatic or intrahepatic cholestasis, inflammation around

the numerous perilobular and intralobular ductules stimulates fiber formation, so that fibrous strands traverse the lobular parenchyma (see Inflammation, under Morphologic Appearance, Chap. 24). Much connective tissue is present in the parenchyma without disturbing the lobular patterns (pseudocirrhosis).

Chemical Measurement and Regression of Fibrosis

In man, no correlation is apparent between the amount of fibrous tissue determined chemically and the weight of the liver in noncirrhotic livers, whereas good correlation is found in cirrhosis [3494]. In experimentally produced cirrhosis, the chemical collagen content rises with the increase in both histologic collagen and reticulum fibers [2351]. The chemical determination is of value in determining regression of fibrosis originally produced by chronic carbon tetrachloride or butter-yellow intoxication. Fibrosis produced by butter yellow almost completely disappears after discontinuing its administration; this is true to a somewhat lesser extent after carbon tetrachloride administration is stopped. The regression is retarded by a high-fat-low-protein diet or bile duct ligation, indicating that it depends upon the regenerative ability of the hepatic cells [2351]. Roentgenologic examinations [3192] confirm the possibility of removal of excessive fibrous tissue suggested by histologic methods [467].

Hepatic cirrhosis, characterized by increase in connective tissue and by parenchymal changes, has important functional consequences, in contrast to simple fibrosis. Whether cirrhosis starts as a primary lesion of the hepatic cells, as most authorities believe [1696], or as a primary mesenchymal process [2797] is still unsettled. Whether the connective tissue alterations, scarring [1497, 1696, 2187], or the parenchymal changes [135, 2337] are more important in the fully developed cirrhotic process is debatable. Inflammation is also considered a significant feature of cirrhosis [2337], and some investigators hold that chronic hepatitis is identical with cirrhosis. Few medical terms have elicited as much semantic discussion as cirrhosis, and many authorities have defined it differently [1696, 1996]. The term "cirrhosis" is derived from the Greek word for yellow, and is, as such, non-committal. It has wrongly been implied to mean hardening of the liver. Extensive fibrosis in the peripheral or central zones may be associated with hardening and granularity and should not be termed cirrhosis. Himsworth wants to discard the term "cirrhosis" and use simply the term "fibrosis," because of the disagreement in defining the lesion [1497]. While no disagreement exists concerning fully developed typical forms, the argument continues as to the initial stages.

Cirrhosis is best characterized by distorted reconstruction of the lobular architecture throughout the entire liver, or at least in a considerable part of it. This implies alteration of both the parenchyma and the framework and agrees with the usual definition of cirrhosis, which was first given by Moon [2337] and was accepted by Karsner [1696]. They consider that cirrhosis is a progressive chronic inflammation, diffuse in extent, accompanied by fibrosis, retrogressive changes in

the parenchymal cells, and regeneration of the remaining cells. The definition of Rössle, representing the German school, lists three factors: (1) destruction of liver tissue; (2) formation of scar tissue; (3) hyperplasia or regeneration [2797]. F. B. Mallory defines cirrhosis simply as a sclerosed condition of the liver.

Distorted reconstruction of the lobular pattern requires further definition. This definition should explain the main functional consequences of cirrhosis, which are (1) manifest degeneration or necrosis of the hepatic cells, or a tendency in this direction, which implies reduced reserve or inferiority of the hepatic cells; (2) portal hypertension as a result of altered intrahepatic blood flow; (3) systemic effects, such as portosystemic encephalopathy [3044], which result from blood bypassing the hepatic parenchyma. These functional effects are caused by regenerative nodules and by vascular anastomoses. These two features are thus the basic features of the distorted lobular reconstruction which characterizes cirrhosis. They are common to all types of cirrhosis, which exists in many etiologic and morphologic forms and which may be brought about by several morphogenetic pathways [33, 1005, 1696, 1996, 2797].

Focal Cirrhosis

The presence of a few foci of abnormal reconstruction does not necessarily justify the term "cirrhosis." Distorted architecture with altered reconstruction may occur in the subcapsular area in an otherwise normal liver, or in the vicinity of trauma or space-occupying lesions, such as tumors or abscesses. In effect, the same pathways may be followed as in diffuse cirrhosis, with similar local consequences. Focal areas of cirrhosis not related to any other abnormalities are ham-

tomas (see Hepatic Hamartomas, Chap. 57), or the result of local disturbances of circulation, of biliary drainage [224], or of external pressure, in which case the picture resembles that of focal postnecrotic cirrhosis. In smaller lesions both possibilities exist. Large nodes are probably hamartomatous. Despite the morphologic similarity to diffuse cirrhosis, focal cirrhosis is clinically important mainly as a possible source of hepatocellular carcinoma in noncirrhotic livers, as a palpatory phenomenon, or as an unexplained nodule seen at laparotomy. Similarly, atrophy of the left lobe of the liver is also associated with focal cirrhotic changes [225].

MORPHOGENETIC PATHWAYS

Several pathways lead to cirrhosis, each of which starts from a different pathologic lesion in the liver. These pathways eventually converge into a final common pathway, and the end stage no longer reveals the morphogenesis. These pathways do not identify the etiology. Different etiologic factors produce the same morphogenetic pathway if they produce the same initial lesion. Moreover, the same etiologic factors may stimulate different pathways, even in animal experiments [1173]. Finally, the same cirrhotic liver frequently reveals indications of different pathways. All this renders the recognition of morphogenetic types of cirrhosis difficult, and any classification is incomplete. The main pathways are collapse, septum formation, and cholangiolar fibrosis. The recognition of the pathways justifies the use of these morphogenetic terms as names of the types of cirrhosis, replacing inaccurate, erroneous, or vague descriptive names or eponyms.

Collapse: Postcollapse or Postnecrotic Cirrhosis

Collapse following massive or submassive necrosis or repeated focal necrosis results in a circumscribed scar, the size of which depends upon the number of lobules involved. This scar, as such, does not represent cirrhosis (Fig. 106A). However, collapse frequently sets in motion alterations of the intervening parenchyma which ultimately lead to cirrhosis. In addition, the underlying disease or other contributing factor, such as malnutrition, may alter the intact parenchyma independently of the collapse and thus contribute to cirrhosis formation.

Processes Damaging Intact Parenchyma. The following abnormal processes account for altera-

tions in the parenchyma near areas of collapse [2630]:

1. Approximation of the portal and central fields causes a rapid transfer of blood from the portal vein branches and hepatic artery branches on one side, to the hepatic vein branches on the other side. This takes place through sinusoids in the collapsed parenchyma which have become transformed into venules (Fig. 109A, B).

2. Rapidly developing collapse produces fissures in the surrounding parenchyma, in which septums form and subdivide the lobules (Fig. 107C).

3. The approximation of the vessels in the portal and central fields produces angulation of the vessels, which disturbs the blood flow.

4. Following submassive necrosis and collapse, lobular remnants varying in size from fragments of lobules to continuous parts of several lobules become unilobular or multilobular nodules (Figs. 90, 108B) (see Formation of Regenerative Nodules, later in this chapter).

5. Collapse occurring in diffuse liver disease is usually associated with extensive regeneration, not only on the edges of the necrotic areas but also within the parenchyma. Nodular regeneration is excessive and may even appear adenomatous. Regeneration remote from necrotic areas is probably due to a humoral effect resulting from disappearance of liver tissue (see Regeneration after Removal of a Large Part of the Liver, under Regeneration, Chap. 13). Nodular or diffuse regeneration interferes with the blood flow through the liver by compression of the connective tissue septums.

6. Portal inflammation, probably caused by hepatic-cell breakdown products, develops in areas of submassive necrosis. It produces periportal necrosis, with subsequent membrane and septum formation leading to subdivision of the lobules.

These six processes leading to cirrhotic transformation depend upon the rate, degree, and distribution of necrosis. Zonal necrosis, even if it develops repeatedly, fails to produce this type of cirrhosis, but frequently repeated focal necroses can produce it, as seen in experimental chronic ethionine intoxication. This indicates that the same may happen in prolonged viral hepatitis in man. Severity and the rapidity of necrosis and collapse are the most important factors. The six processes are absent or progress slowly in gradually developing collapse. Postnecrotic scarring

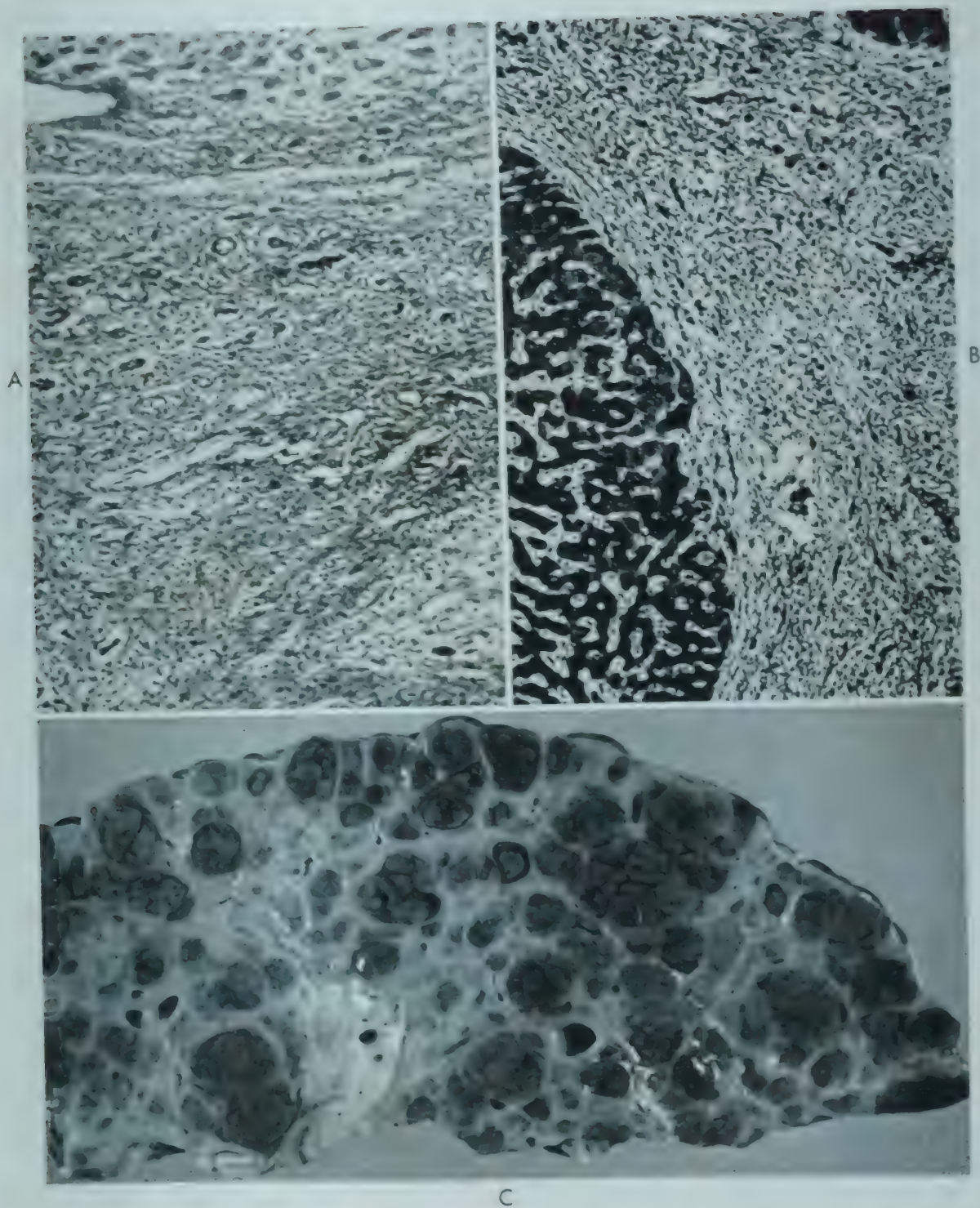


FIG. 109 A. Broad connective tissue septum as end result of collapse. It contains many veins. They are transformed from sinusoids of the lobular parenchyma, which has disappeared. Van Gieson's (×95). (Popper, H., Elias, H., and Petty, D.: *Am.J.Clin.Path.* 22: 717, 1952. courtesy of The Williams & Wilkins Company.) B. Lobular remnant showing regeneration bordering on a collapsed zone containing persisting vessels and proliferating ductules in postnecrotic cirrhosis. Mallory's aniline blue (×90). C. Broad tissue bands consisting of collapsed framework separating nodules composed of persisting and regenerating parenchyma, in postnecrotic cirrhosis (Marchand's coarse nodular hyperplasia).

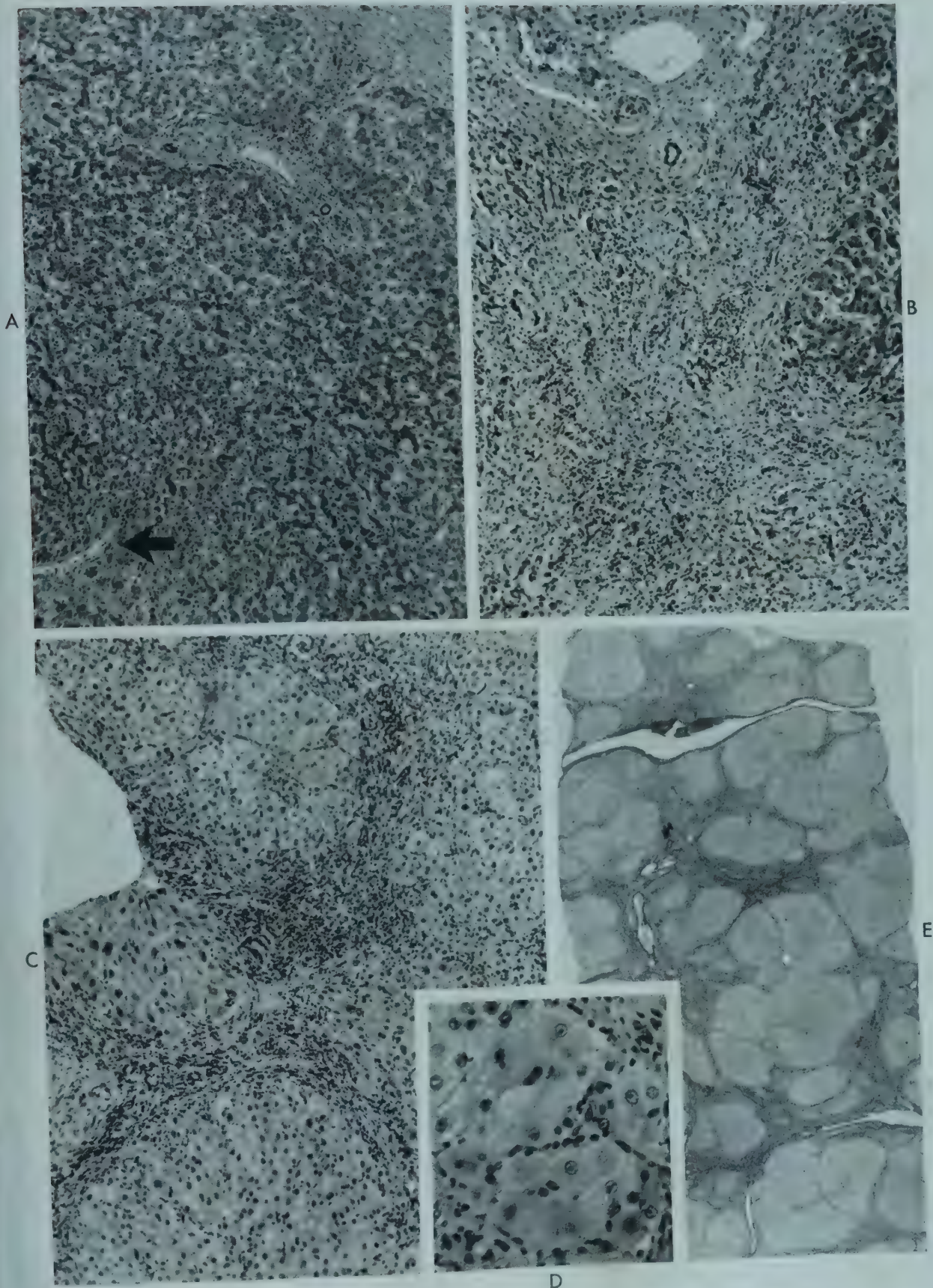


FIG. 110 A. Postnecrotic cirrhosis with intact lobular architecture in the nodule. See portal vein and central vein (indicated by arrow) and collapse in tissue surrounding the nodule. H&E ($\times 50$). B. Broad connective tissue band in postnecrotic cirrhosis. It contains approximated vessels and accumulations of ductules surrounded by scavenger cells. These accumulations indicate the preformed portal tracts. H&E ($\times 70$). C. Biopsy specimen of postnecrotic cirrhosis following viral hepatitis. Bizarre regeneration with multinucleated giant cells in the regenerative nodules. H&E ($\times 100$). [See insert D at higher magnification ($\times 300$).] E. Postnecrotic (coarse nodular) cirrhosis. The large nodules consist of many lobules. Van Gieson's ($\times 3$).

without functional significance is seen in syphilitic *hepar lobatum* (see *Hepatic Gummas and Hepar Lobatum*, under *Hepatic Syphilis*, Chap. 54), in atrophy of the left lobe of the liver (see *Local Atrophy*, under *Atrophy*, Chap. 22), or in rare cases of so-called coarse nodular hyperplasia, in which the parenchyma between the scars is little altered. The term "cirrhosis" does not apply in these instances, either from a functional or from a morphologic standpoint. However, in the majority of instances in which massive or submassive necrosis develops in diffuse liver diseases, the speed and extent of the necrosis are sufficient to produce cirrhotic changes in the surrounding parenchyma. There therefore appears to be justification for separation of the consequences of the collapse into innocuous postnecrotic scarring as distinguished from postnecrotic or postcollapse cirrhosis, which exhibits the structural and functional changes in the surrounding parenchyma. Since necrosis may occur in many other types of cirrhosis, replacement of the term "postnecrotic" by the term "postcollapse" might be advantageous. In view of the long established usage of the term "postnecrotic," it is retained here.

Depending on the speed and degree of the initial collapse process, the appearance varies from a liver with a few scars and large nodules to a fine nodular, almost diffuse cirrhosis. Little agreement on criteria for the definition of postnecrotic cirrhosis can be found [135, 1201, 1696].

Secondary Collapse. Secondary collapse in a cirrhotic liver leaves its imprints as postnecrotic changes in other types of cirrhosis, in contrast to primary collapse in a previously normal liver. These changes are coarse nodules and broad connective tissue bands. Moreover massive and submassive necrosis may be present, thereby resulting in a mixed type of cirrhosis, depending upon the extent of necrosis and the admixture of other components.

Morphology. Grossly, broad connective tissue bands, in which vessels are recognized, alternate with narrower bands (Fig. 109C). The nodules formed by these bands vary in size in the same liver, as well as in different ones, and may be larger than 1 cm in diameter. The left lobe is usually more involved and may even become completely atrophic [1497]. Microscopically, the irregular distribution and the great variation in the size of the nodules are the most outstanding features (Fig. 110E). Many portal tracts and central fields appear normal, particularly in the

larger nodules (Fig. 110A). The lobular architecture in these areas is intact because the original lobules were preserved as large multilobular nodules or because new lobules were formed. In the intervening bands, the vessels are approximated, ductules increased, and many scavenger cells found (Fig. 110B). Other portal tracts and central fields are altered by the presence of thick or thin fibrous bands, the largest ones being patchy areas of massive collapse. The differences in distribution of vessels and the texture of membranes in primary and secondary collapse were referred to above. Bizarre irregular regeneration of hepatic cells or in the nodules (Fig. 110C, D) is fairly characteristic. This is especially helpful in biopsy specimens, in which the nature of the bands can not be evaluated because of the small size of the sample. Sparsity of focal necrosis with segmented leukocytes is not a constant criterion.

Nomenclature. Variations in the picture have resulted in a number of different names, such as "coarse lobular cirrhosis" of Klemperer, "toxic cirrhosis" of Mallory [2187], "healed acute atrophy" of Goodpasture, "postnecrotic cirrhosis" of Karsner [1696], "postnecrotic scarring" of Himsworth [1497], "multilobular cirrhosis" of Sabourin, and Marchand's "cirrhosis." In the United States, the lesion often follows severe viral hepatitis with massive necrosis, but chemical poisons can not be excluded, and frequently the etiology can not be determined. In the tropics it is seen as a result of malnutrition.

In animals, postnecrotic cirrhosis has been produced after massive necrosis on a clearly defined dietary basis [707, 1193, 1320, 1497]. Coarse nodular cirrhosis has been found after prolonged ethionine intoxication in rats following diffuse but not massive necrosis.

Primary Septum Formation (Septal Cirrhosis)

In various diffuse liver diseases, septums form without preceding significant collapse, either within the parenchyma or originating in central or portal canals. They result from condensation of membranes, and eventually they subdivide the lobules. Since few, if any, lobules are spared in diffuse diseases, the uniformity of septum formation is characteristic of this type of cirrhosis.

Mechanism of Septum Formation. Collagenous membranes, occasionally reinforced by fibers, may condense to form septums. In radiating types of central (Figs. 108C, 111B) or peripheral (Fig.

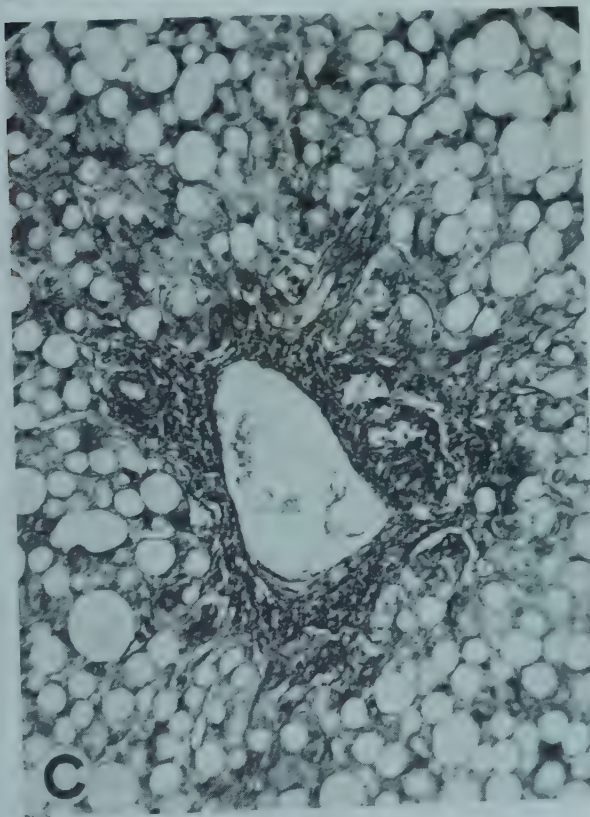
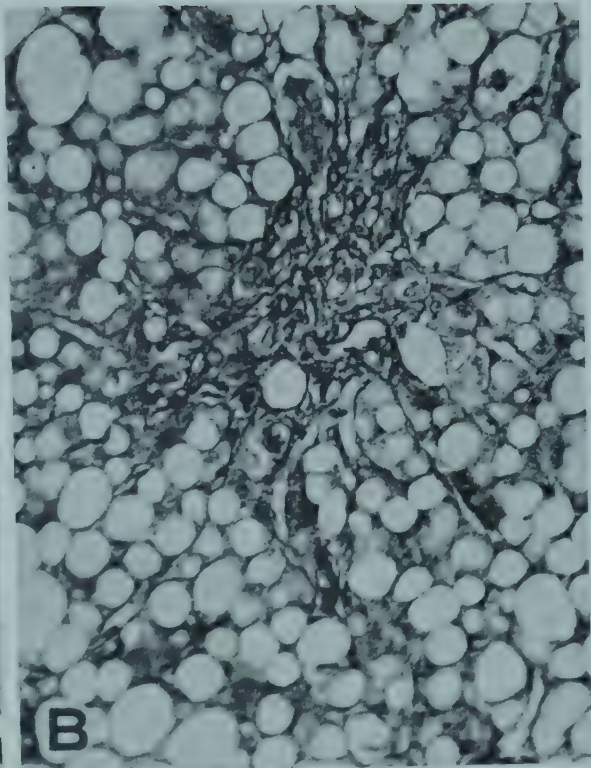
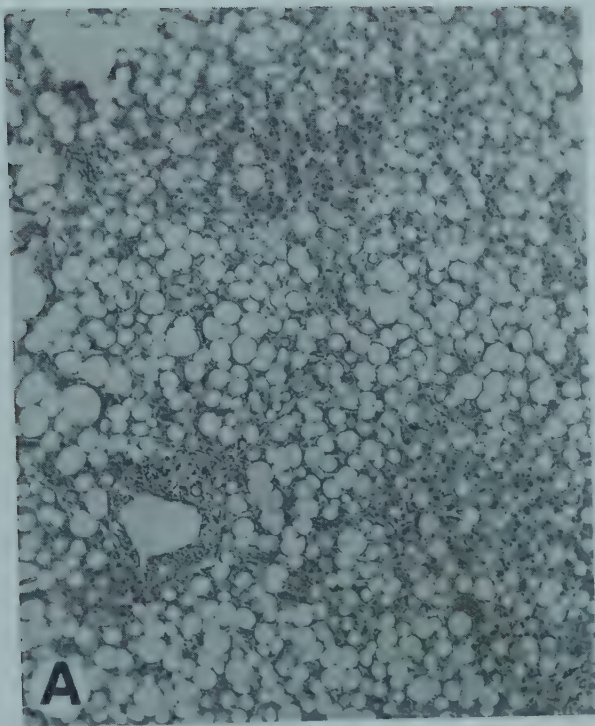


FIG. 111 A. Transition of fatty liver into cirrhosis I. Fatty liver with entirely undisturbed lobular architecture; slight enlargement of the portal tract containing inflammatory exudate extending strandlike into the parenchyma. H&E ($\times 57$). B. Collagenous membranes extending from a central canal into the surrounding parenchyma. Mallory's aniline blue ($\times 180$). C. Stellate appearance of portal tract produced by radiation of collagenous membranes into surrounding parenchyma; disruption of limiting plate. Mallory's aniline blue ($\times 180$). D. Beginning formation of septums which extend from central and portal canals. Mallory's aniline blue ($\times 49$). (Popper, H., Szanto, P. B., and Elias, H.: *Gastroenterology* 28:183, 1955; courtesy of The Williams & Wilkins Company.)

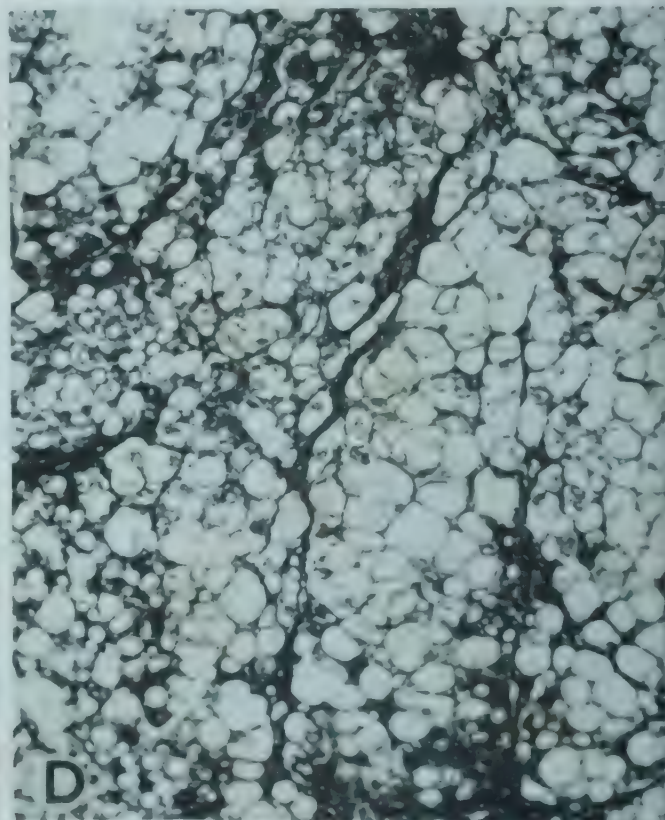
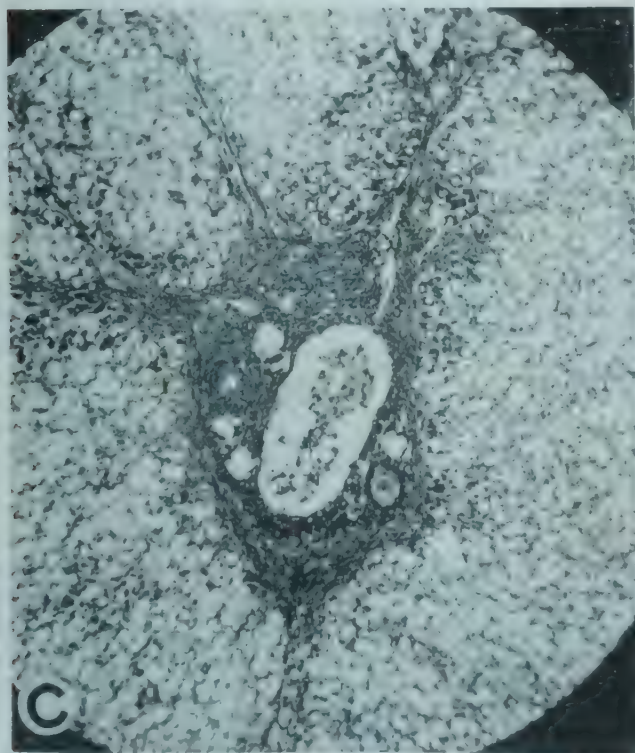
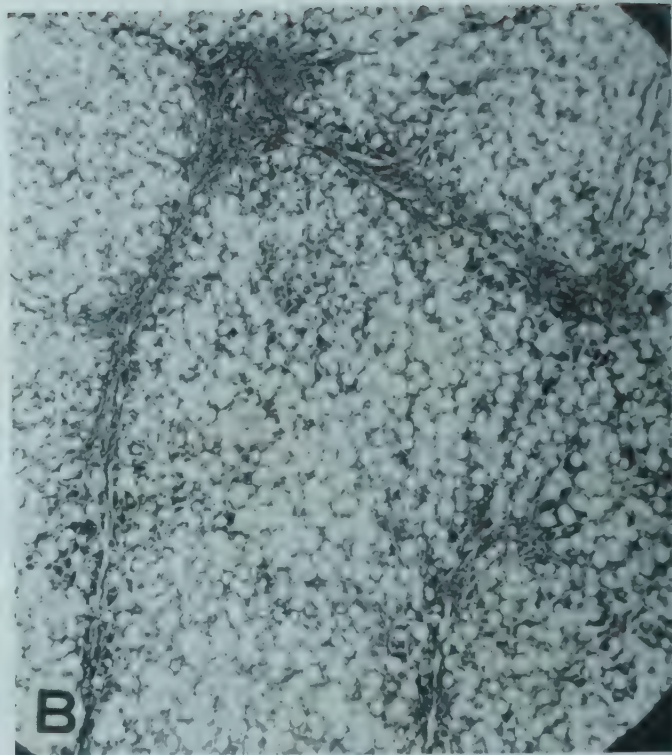
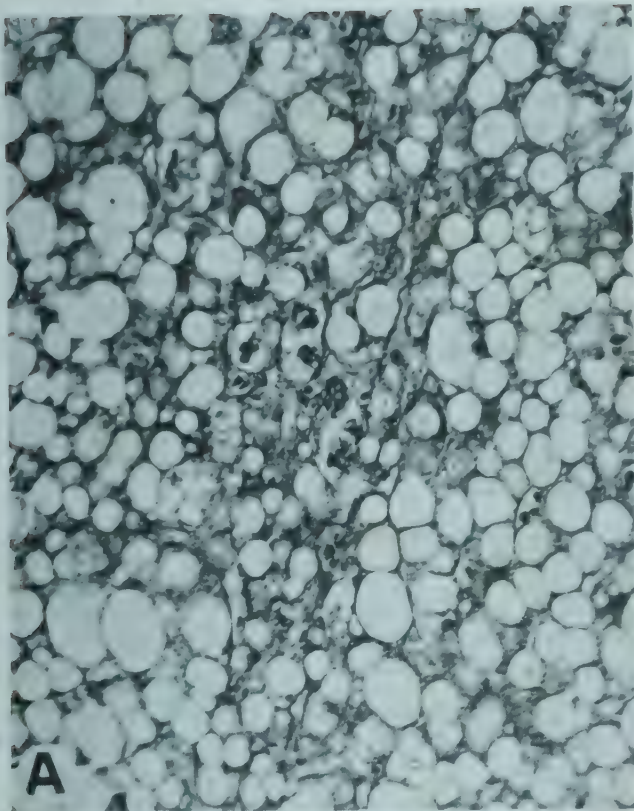


FIG. 112 Transition of fatty liver into cirrhosis II. Mallory's aniline blue. A. Membranes developing within the lobular parenchyma around focal necrosis ($\times 190$). B. Septums connecting portal tracts, producing perlobular fibrosis ($\times 52$). C. Septums extending into the lobular parenchyma from portal canal ($\times 40$). D. Straight septums separating territories of different fat content without relation to lobular topography ($\times 110$). (Popper, H., Szabo, P. B., and Elias, H. *Gastroenterology* 28:483, 1955; courtesy of The Williams & Wilkins Company.)

8D) membrane formation, for example, some of the membranes are selected to form septums (Fig. 111D). The reasons for this selection are usually not apparent but are probably related to blood flow and local oxygen supply. The hepatic cells between membranes forming the septum disappear, while some of the sinusoids persist and even assume the appearance of veins. Such septums extend into the lobular parenchyma, usually in the form of straight sheets with at least one free edge, and appear to be straight lines in histologic sections (Fig. 112B and D). Similar septums develop from intralobular membrane formation around foci of necrosis, granulomas, or areas of regeneration (Fig. 112A). Another source of irregularly arranged septums is the stress fissures previously discussed.

Septums traverse the lobules in different planes in three-dimensional reconstructions (Fig. 113A). They may meet, or a septum extending from the portal field may progress to a central field, or vice versa (Fig. 113B). These connections result in subdivision of the lobule. The fragments of the lobules after this subdivision become nodules, with loss of the lobular architecture. The subdivision of the lobule by septums varies from bisection of a lobule to extensive fragmentation. Septum formation is the basic process which morphogenetically characterizes septal cirrhosis.

Peripheral Septum Formation. Peripheral membrane formation, with subsequent septum formation and connections between portal and central

fields, results from various inflammations in the portal tracts. These inflammations include the irritating effects of iron deposition in hemochromatosis, purulent inflammation in infected biliary hepatitis, and occasionally some granulomas, such as those produced by various microorganisms. Peripheral septum formation is also seen in chronic viral hepatitis, but the progression to septal cirrhosis in the absence of other causative agents has not yet been proved.

Central Septum Formation. Central membrane formation in chronic passive congestion [2359] or in repeated toxic injuries with central necrosis produces septums starting from the center. Extension of these septums with the development of portocentral connections leads to relatively rare types of septal cirrhosis. One example is cardiac cirrhosis; another is the cirrhosis which occurs in hyperthyroidism [2798], although in some cases the process seems to start from the lobular periphery [2359].

Septum Formation in Fatty Metamorphosis. The nature of the transformation of the fatty liver into cirrhosis is a problem for which many theories have been advanced. The previous assumption that fatty livers necessarily become cirrhotic is now challenged. Various mechanisms have been suggested for this transformation, such as interference with intralobular circulation [640, 1193, 1497]. In animals with cirrhosis produced by high-fat-low-protein diets or carbon tetrachloride poisoning, impaired blood flow in the

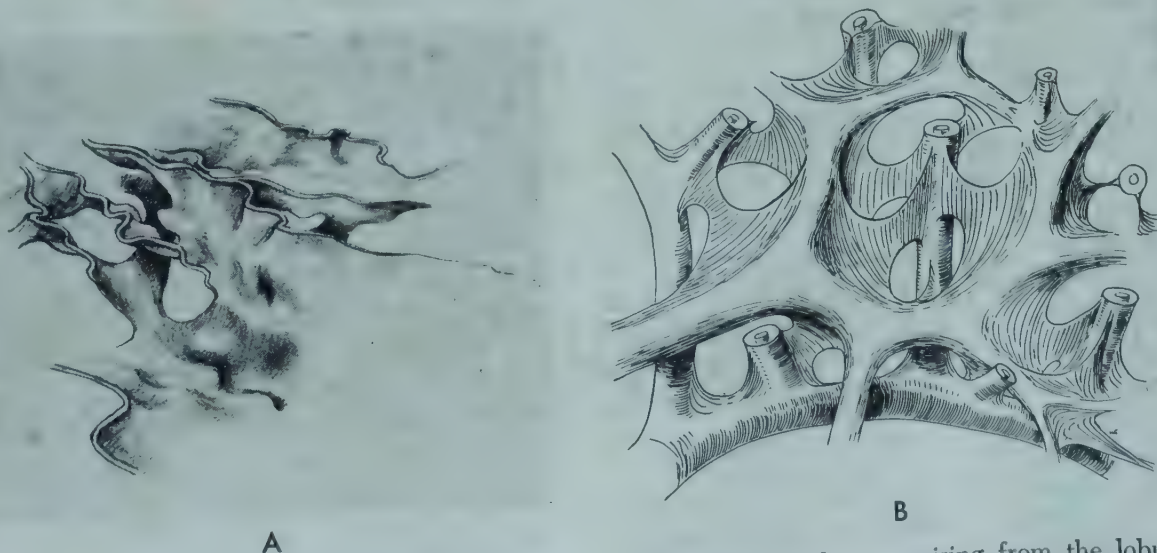


FIG. 113 A. Three-dimensional reconstruction of collagenous membranes arising from the lobular center, in cirrhosis. (Elias, H., in *Liver Injury*, Tr. Eleventh Conf., 1952, New York, Josiah Macy, Jr. Foundation.) B. Schematic drawing of septums extending between the portal and central canals, which cross in three-dimensional space. (Popper, H., in *Liver Injury*, Tr. Eleventh Conf., 1952, New York, Josiah Macy, Jr. Foundation.)

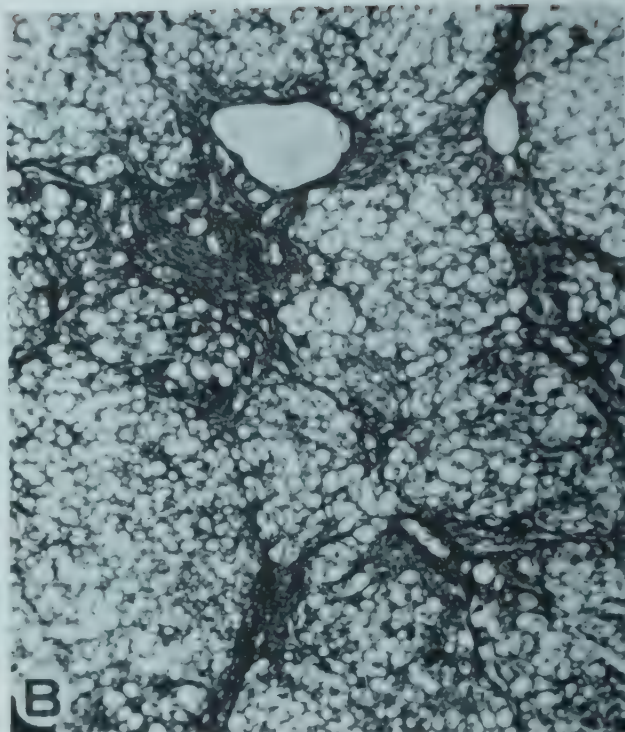
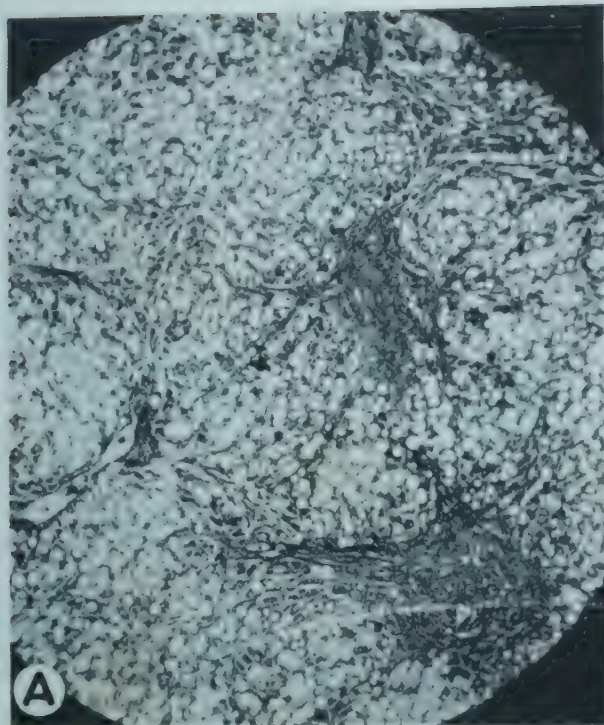


FIG. 114 Transition of fatty liver into cirrhosis division of the lobules ($\times 40$). B. Completion of lobular into nodular architecture ($\times 52$). (Popper, H., Szanto, P. B., and Elias, H.: *Gastroenterology* 28: 183, 1955; courtesy of The Williams & Wilkins Company.)

III. A. Connection of septums with beginning sub-septum formation with transformation of the lobular into nodular architecture (Figs. 111D, 112C).

sinusoids has been suggested on the basis of injection preparations [1193]. The development of cirrhosis in man or animals resulting from hepatic infiltration with cholesterol, glycogen, polyvinyl alcohol, and even silica gels is also said to be caused by vascular interference [1194, 3439].

The peculiar vascularization of the liver in rats (see Chap. 17) (Fig. 59) makes it appear possible that fatty metamorphosis as such induces cirrhosis. Fatty cysts form in the central zone and also around middle-sized portal tracts [1406]. The parenchyma around these tracts is functionally "nonportal," since they receive their blood from remote portal vein branches. Collapse of the cysts leads to septums connecting central canals with larger portal tracts and subdivision of the lobule. In man, the parenchyma around portal tracts is "portal," and this mechanism is of little significance. Observation of transitional stages in man and three-dimensional reconstruction studies indicate four types of septums transforming the fatty liver into cirrhosis (Figs. 111, 112, 114):

1. Central septums. These form around fatty cysts and follow central membrane formation. The septums extend from the center into the lobular parenchyma, possibly through territories of lower oxygen saturation (Fig. 111B).

2. Peripheral septums. Peripheral and periportal

inflammation, often associated with fatty metamorphosis, results in peripheral membrane formation and stellate enlargement of the portal tract (Fig. 111C). In later stages, perilobular fibrosis arising in the forks between branching portal canals (Fig. 112B) is followed by septums extending into the lobular parenchyma (Figs. 111D, 112C).

3. Intralobular fibrosis. This develops around areas of focal necrosis or regeneration (Fig. 112A).

4. Septums in stress fissures between territories of uneven tissue turgor. Their direction is not related to lobular topography and they appear as straight lines in sections (Fig. 112D).

Eventually the various septums meet and subdivision of the lobule begins (Fig. 114A). Subsequently, the lobular parenchyma is transformed into a nodular parenchyma (Fig. 114B). The contributions of the different types of septums vary in each instance. Only the first form, the least frequent in man, is caused directly by fat, while the others are the result of inflammation, necrosis of hepatic cells, and variation of the fat content in time and space.

Periductular Fibrosis; Pseudocirrhosis

Inflammation along the perilobular and intralobular ductules and lymph vessels (see Portal

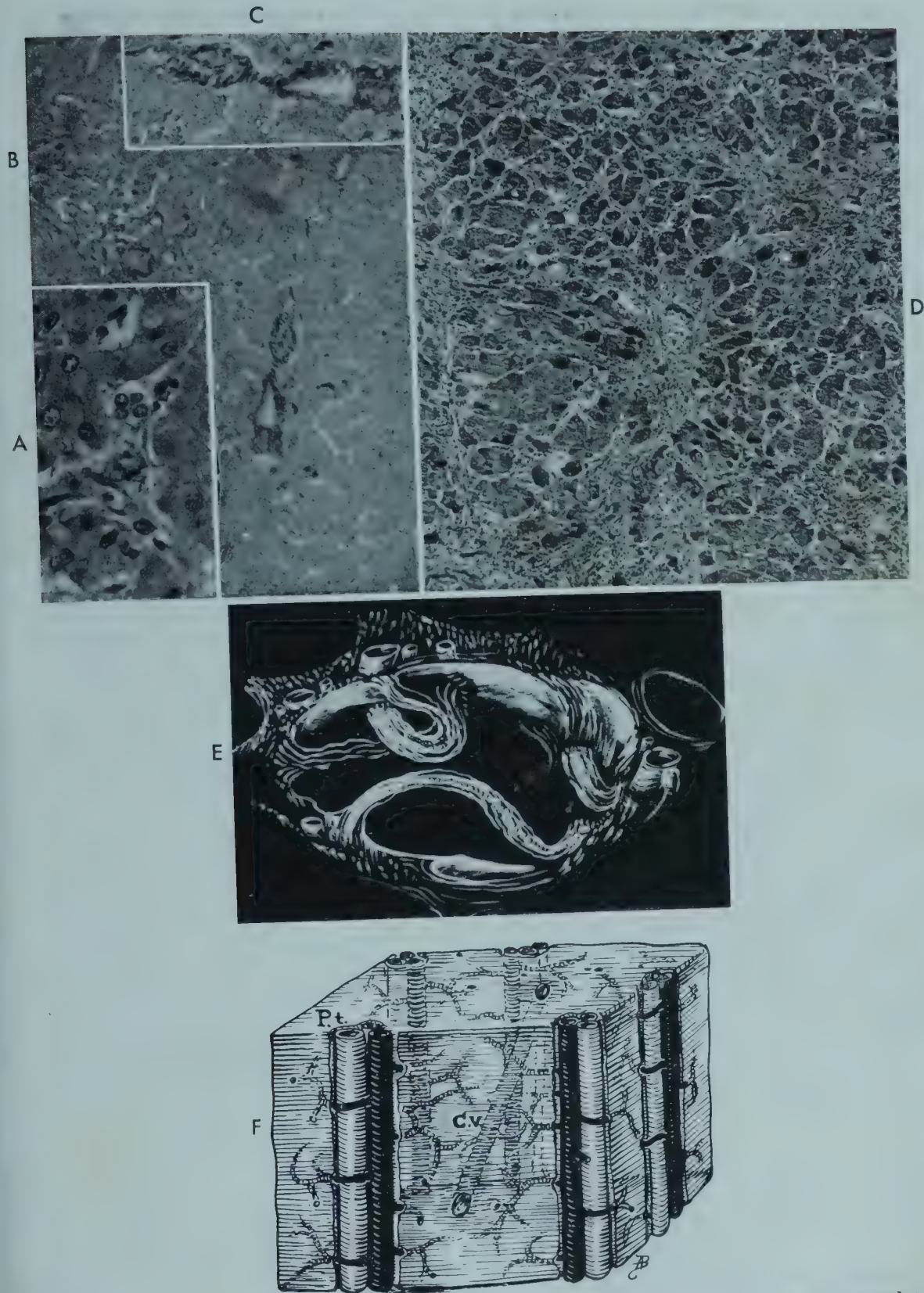


FIG. 115 A. Large intralobular bile ductule accompanied by an arteriole, both invested in a common adventitia. H&E ($\times 210$). B. Intralobular cylindriform fibrosis ensheathing ductules with pericholangiolitic inflammation. Note elliptic shape of section of fibrosis, which is different from that of septums. Van Gieson's ($\times 54$). C. Detail of 115B. Oblique section of cholangiolitic fibrosis, showing oblique and transverse sections of fibers. Van Gieson's ($\times 210$). D. Cholangiolitic cirrhosis with maintenance of lobular architecture, the parenchyma being traversed by cylindriform fibrosis. H&E ($\times 54$). E. Reconstruction of curved and cylindrical areas of fibrosis in cholangiolitic cirrhosis. F. Drawing showing the lobules traversed by increased and thickened cylindrical areas of fibrosis containing ductules. The lobular pattern is not disturbed. (Popper, H., and Elias, H.: *Am.J.Path.* 31:405, 1955.)

and Periportal Inflammation, under Focal Necrosis, Chap. 25) may result in fibrosis in and around the periportal and intralobular trabeculae containing bile ductules, arterioles, and lymphatic vessels [1160]. Since perilymphangitis usually does not last long enough to produce much fibrosis, intrahepatic cholestasis, or "cholangiolitis," and infected extrahepatic cholestasis are more common causes of this type of fibrosis. Whatever the etiology, periductular fibrosis forms strands which traverse the lobules as a network of varying density (Fig. 115*B, C, D*). These strands are composed of fibers, not of membranes. In three-dimensional models they appear to be cords which do not disturb the lobular blood flow, since the lobule is not dissected (Fig. 115*E, F*). In this stage, regenerative nodules and altered reconstruction are not present, and the term "pseudocirrhosis" is recommended. The strands become connected with each other only in advanced stages by sheetlike connective tissue septums, especially if prolonged inflammation causes radiating membrane formation from the portal tracts and the fibrous strands into the lobular parenchyma (Fig. 115*F*). This secondary septum formation justifies the inclusion of this process under cirrhosis. Portal hypertension develops, and the clinical and pathologic manifestations of the late stages of pseudocirrhosis are indistinguishable from those of septal cirrhosis [3510]. Pseudocirrhosis from intrahepatic cholestasis or "cholangiolitis" is probably the most common precursor of diffuse intralobular cirrhosis of Hanot. Diffuse intralobular inflammation, or diffuse infiltrative interstitial hepatitis [3642], may progress to intralobular fibrosis and septal cirrhosis without an intermediate stage of pseudocirrhosis.

PROCESSES COMMON TO ALL TYPES OF CIRRHOSIS

Two main processes are common to the three morphogenetic types of cirrhosis, namely, nodule formation and vascular anastomosis.

Formation of Regenerative Nodules or Pseudolobules. One basis of altered reconstruction is the regenerative or hyperplastic nodule characteristic of cirrhosis [1400]. It differs from the lobule by the absence of a definite central vein, although the hepatic-cell plates converge toward the center of the nodules, which can be injected from the hepatic veins [2631] (Fig. 116). However, the nodules are eccentrically located with regard to

the original central vein. The contribution of the hepatic artery to the efferent blood supply of the nodular parenchyma appears to be greater than to that of the normal lobular parenchyma. The reticulum framework of the nodule does not differ from that of the lobule. The nodule develops from lobular fragments in several ways.

1. In submassive collapse fragments of different size persist and regenerate (Figs. 90, 107*D*), with new formation of reticulum framework and of sinusoids and possibly even new portal tracts.

2. The lobule can be subdivided by septums, especially if they link central and portal canals (Fig. 113*B*). Since central and portal canals cross in space, the septums connecting these canals dissect irregularly shaped portions from these lobules.

3. Isolated cells may persist in periportal necrosis, trapped in the surrounding connective tissue (Fig. 117*A*). They may proliferate to become nodules, with new formation of reticulum framework and sinusoids (Fig. 117*B*).

Whenever a lobular fragment becomes separated from the rest of the parenchyma, the hepatic-cell plates are rearranged so that they radiate from the center of the nodule under the influence of the direction of the blood flow. Regeneration is humorally stimulated by the loss of hepatic cells throughout the liver, and all the cells in the nodule participate in this, but it is most prominent on the periphery of the newly formed nodule. The hepatic-cell plates become two or more cells thick, as in an embryonal liver (Fig. 67), and small nodules or the periphery of large nodules appear as blastemas (Figs. 116, 117*C*). This regeneration may stop, and the nodule becomes mature, with one-cell-thick plates. In other instances circulatory difficulties arise in the centers of the nodules because of growth and the shunting of blood away from the nodule by portohepatic venous anastomoses (see Vascular Anastomoses, next heading). Central and submassive necrosis results, with almost complete destruction of the nodule and secondary collapse. The remaining nodular fragments give rise to new nodules, and thus a never-ending cycle of nodule formation is set in motion, which is characteristic of active and progressive cirrhosis. Some nodules consist of several lobules with only slightly distorted lobular architecture. This is partly caused by regenerative new formation of portal tracts and mainly by persistence of whole lobules or confluent parts of

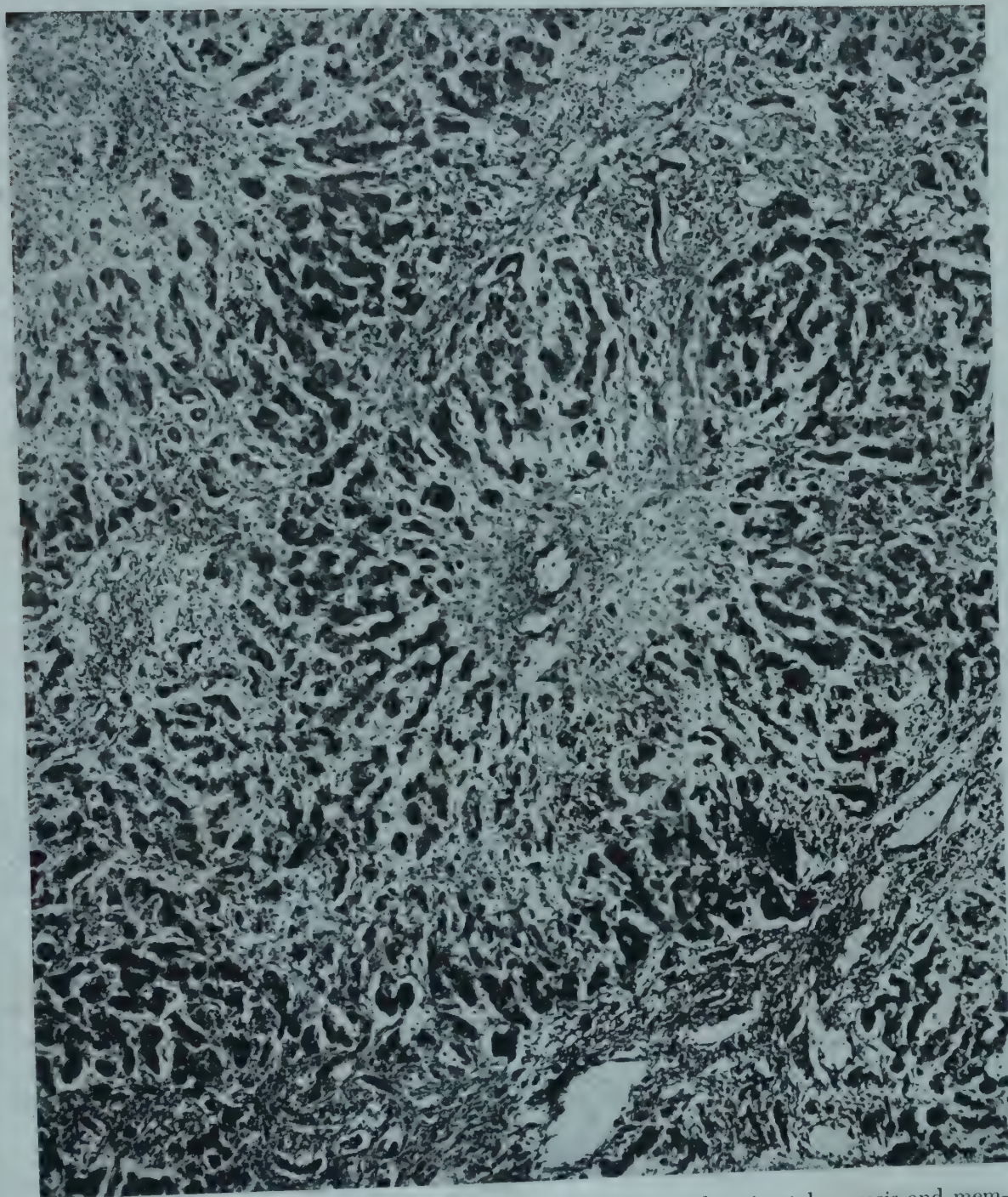


FIG. 116 Subdivision of lobule by septums following central and periportal necrosis and membrane formation caused by both chronic passive congestion and portal inflammation. H&E ($\times 75$). (Popper, H., and Elias, H.: *Am.J.Path.* 31:405, 1955.)

lobules after submassive necrosis. These nodules also show intralobular peripheral regeneration and are usually oddly shaped, often having a garlandlike form.

The regenerative nodules exert pressure which is apparent in casts of vessels injected with plastic. In cirrhosis a grossly irregular arrangement is noted (Fig. 118, upper). The portal vein branches form baskets around the nodules, whereas the hepatic vein branches appear flattened, since they are not so well protected by

surrounding connective tissue with bile ducts and arteries as are the portal vein branches (Fig. 117D) [1724, 2631]. As a result of these processes the hepatic venules in injection preparations appear very much distorted and obliterated, with consequent reduction of blood flow. In the portal veins similar changes are present but less conspicuous [2164].

The obstruction of the hepatic veins by the regenerative nodule is considered one of the main causes of portal hypertension. The smaller the

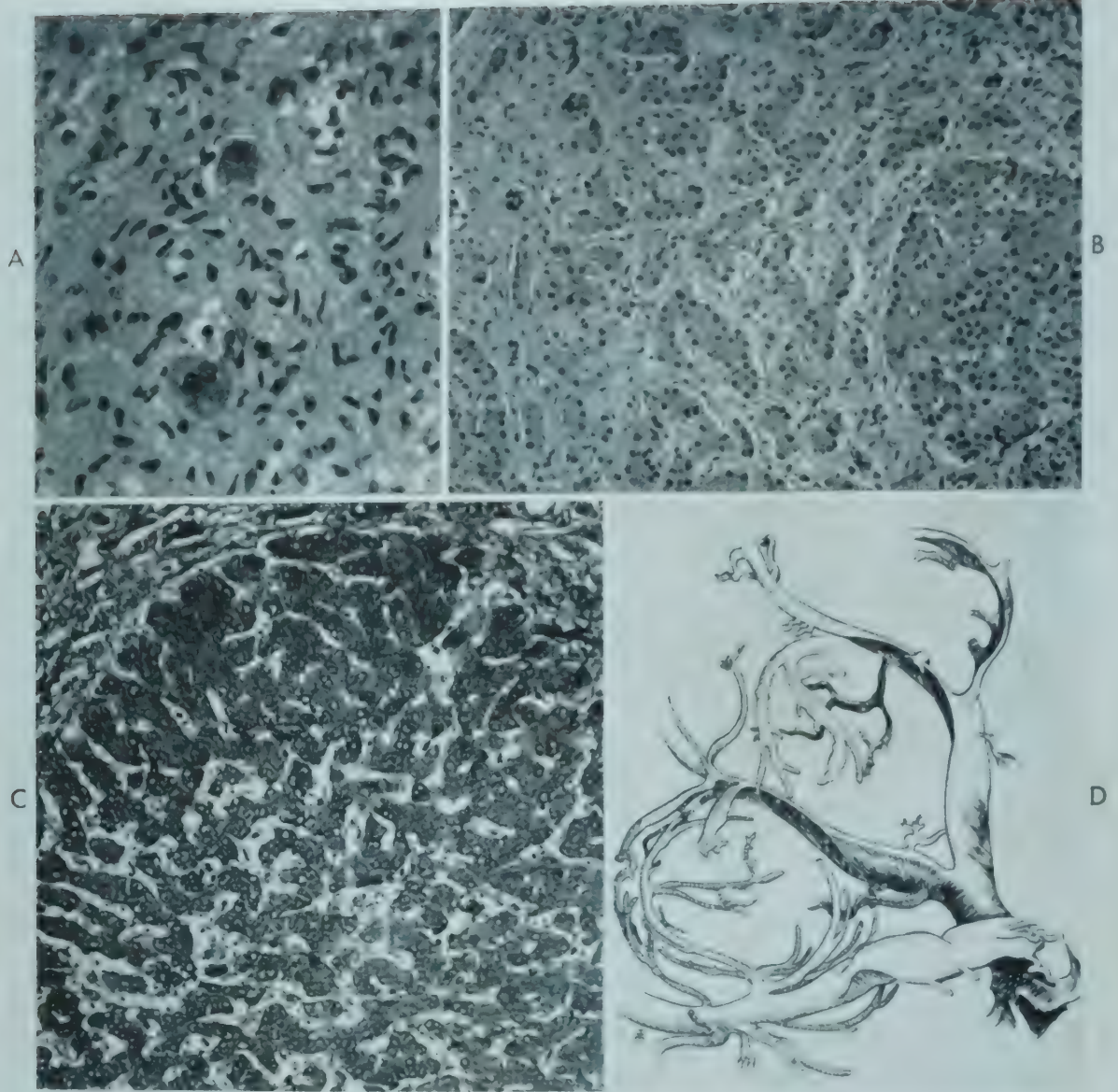


FIG. 117 A. Single hepatic cells trapped in septum in juvenile cirrhosis. H&E ($\times 230$). B. Isolated hepatic cells and several-cell-thick plates in septum of juvenile cirrhosis. Beginning transformation into a nodule. H&E ($\times 130$). C. Large regenerating nodule in septal cirrhosis compressing the surrounding connective tissue and showing one-cell-thick plates in the center and several-cell-thick plates on the periphery. H&E ($\times 90$). D. Basket formation of portal vein branches and flattening of tributaries of the hepatic vein, as drawn from injection preparation in septal cirrhosis. (Popper, H., Elias, H., and Petty, D.: *Am.J. Clin.Path.* 22:717, 1952; courtesy of The Williams & Wilkins Company.)

nodule, the greater the tendency toward portal hypertension because of more extensive venous obstruction [135]. In cirrhosis with ascites this compression is especially severe [565]. The growing regenerative nodule also exerts some pressure on the surrounding septums, which may be bent, weakened, or interrupted by these forces. The surrounding connective tissue framework is compressed [2463], preventing reexpansion. It subsequently becomes collagenized, and thus nodulation results in fibrosis.

These observations give the regenerative nodule great importance in the focal increase of con-

nective tissue, in the distortion of the vasculature, and in the development of portal hypertension. Only exceptionally in areas of massive collapse can a similar distortion of the venous bed caused by fibrosis be observed in injection preparations. In these instances the same sequelae develop in the absence of regenerative nodules. Although fibrosis has been ascribed major significance in distorting the vasculature [1653], it is only locally that it is more important than regenerative nodules.

Vascular Anastomoses. In the formation of septums, as well as during collapse, abnormal

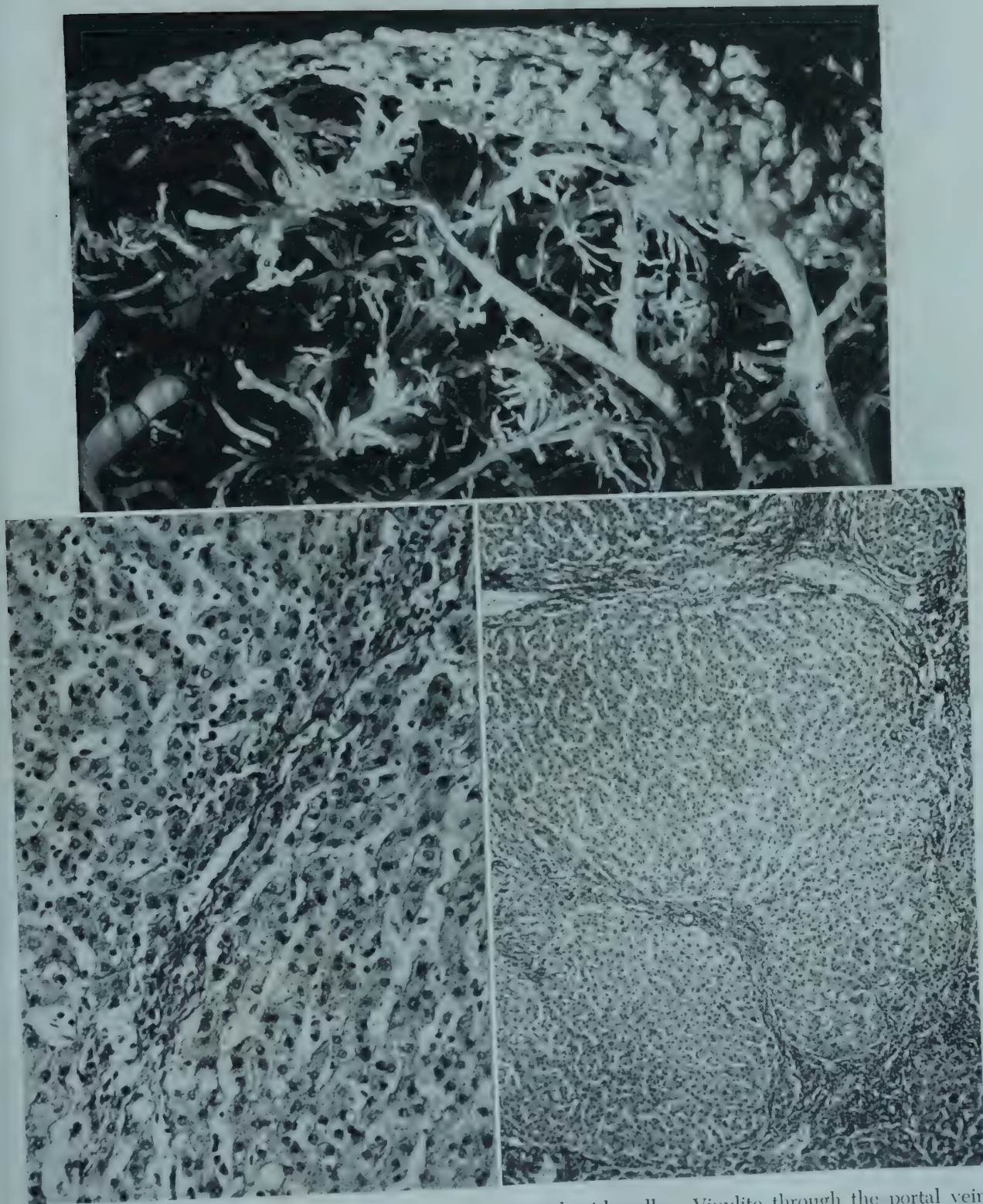


FIG. 118 Upper. Cast of human septal cirrhosis injected with yellow Vinylite through the portal vein and with blue Vinylite through the inferior vena cava. Lower left. Sinusoids included in newly formed septum in septal cirrhosis, while surrounding hepatic cells disappear. Van Gieson's ($\times 243$). Lower right. Central and portal canals connected by septums containing vessels in septal cirrhosis. Van Gieson's ($\times 28$). (Popper, H., Elias, H., and Petty, D.: *Am.J.Clin.Path.* 22:717, 1952; courtesy of The Williams & Wilkins Company.)

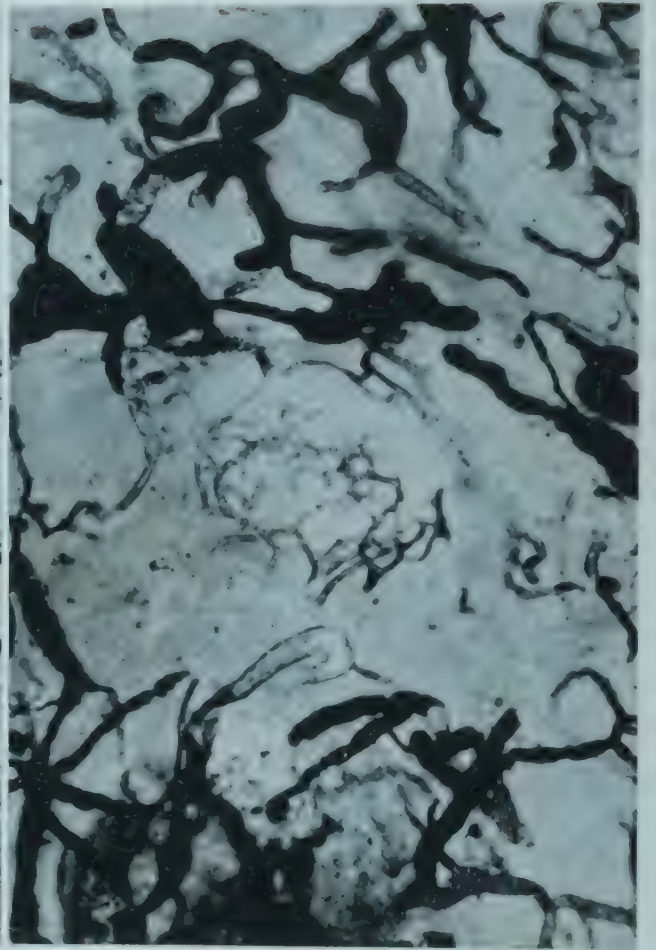


FIG. 119. Upper left. Thick section injected with Berlin blue gelatin (appearing gray) through the portal vein, and with black India ink through a hepatic vein in postnecrotic cirrhosis with extensive nodule formation. The septum contains a dense network of vessels injected through the portal and

ascular communications develop by transformation of sinusoids into venules. In postnecrotic collapse some of the sinusoids serve as portohepatic anastomotic channels between portal and hepatic vein branches, while the rest of the sinusoids are obliterated. Similarly, in septum formation some preexisting sinusoids are selected for blood flow and are thereby transformed into veins, while the remainder become obliterated (Fig. 118, lower left and right). In general, these communications develop from preformed vessels, and the claim that such bivenous strands result from newly formed vessels (angiogenesis) owing to preceding granulomatous inflammation [2359] is true only in the minority of cases. In livers injected from the various vessels with different colored mixtures, an extensive vascular network is noted in the septums [2126] (Fig. 119, upper left). Much blood is in the septal network, whereas relatively little is found in the lobular or nodular sinusoids (Fig. 119, upper right). In this network, branches of the portal and hepatic veins freely anastomose (Fig. 119, lower left and right). These internal Eck fistulas are more than $100\ \mu$ in diameter only in the vicinity of the portosystemic anastomoses near the coronary ligament; they are less than this size in the rest of the liver [2631].

Branches of the hepatic artery also contribute to this vascular network in the septums, communicating freely with portal and hepatic vein branches, as seen in injection preparations [2877, 2931]. In cirrhosis the normal predominance of portal vein branches is no longer apparent in injection preparations, because either the number of arterial branches is increased, or the number of venous branches is decreased, or both.

FUNCTIONAL SIGNIFICANCE. These portohepatic venous communications are absent in the normal livers of most animals, and if they are present they are quantitatively and therefore functionally not significant. In the cirrhotic liver the extensive anastomoses shunt blood directly from the portal

vein into the hepatic vein, bypassing the lobular parenchyma. This bypass produces a circulatory disadvantage to the parenchyma. The functional significance is indicated by the formation of urate stones in the urinary tract of rats with chronic carbon tetrachloride intoxication, after portohepatic anastomoses have developed, and the uric acid escapes transformation in the liver [3389]. Bypass of the lobular parenchyma in human cirrhosis is suggested by reduced Bromsulphalein extraction, for instance, in the absence of demonstrable hepatocellular damage (see Circulatory Insufficiency, under Bromsulphalein Retention Test, Chap. 37). Together with the extrahepatic collaterals, the intrahepatic venous anastomosis in cirrhosis thus reduces hepatic function on merely a circulatory basis, without hepatocellular damage. In the presence of well-functioning hepatic parenchyma, apparent hepatic failure can thereby be severe and even fatal.

The circulatory disadvantage produced by the bypass also disturbs the nutrition of the hepatic parenchyma and is probably responsible for the hypoxic central necrosis characteristically found in cirrhosis. Central necrosis with subsequent collapse results in further progression of the cirrhotic process after the original cause, such as fatty metamorphosis or viral hepatitis, has disappeared [1400]. Development of these anastomoses thus leads to the irreversible stage of cirrhosis [2631]. The anastomoses do not relieve portal hypertension, since the hepatic veins are compressed by regenerating nodules heartward to the anastomoses.

HEPATIC ARTERY-PORTAL VEIN COMMUNICATIONS. Enlargement of presinusoidal communications between the hepatic artery and portal vein (see Structural Principle of the Vascular Tree, Chap. 17) results from the increasing resistance to portal flow by fibrosis, so that relatively more of the blood flowing to the liver is carried by the hepatic artery [2126]. The more advanced the cirrhosis, the more numerous the arterial branches

hepatic veins. The periphery of the nodules is connected with portal branches, the center with hepatic tributaries ($\times 20$). *Upper right.* Same liver, showing dense network of vessels in the septums. Afferent vessels of nodule cut tangentially are very narrow ($\times 100$). *Lower left.* Same liver, showing extensive anastomoses between branches of portal and hepatic veins located in the septums ($\times 150$). *Lower right.* Thick section injected with opaque red ink (appearing white) through the portal vein and with india ink through a hepatic vein in septal cirrhosis, showing extensive anastomoses between branches of portal and hepatic veins located in the septums. Combined transmitted and incident lighting ($\times 200$). (Popper, H., Elias, H., and Petty, D.: *Am.J.Clin.Path.* 22:717, 1952; courtesy of The Williams & Wilkins Company.)

in the septums. The extensive arteriovenous communications and increased arterial blood flow have been considered as one important cause for portal hypertension.

CLASSIFICATION OF CIRRHOSIS

The difficulties in the classification of cirrhosis are indicated by the many and different classifications presented by various authors such as Karsner [1696], Mallory [2187], Rössle [2797], and others. A mere descriptive classification takes into account such qualities as size of the liver (hypertrophic or atrophic), size of nodules (granular, nodular, or coarse nodular), number of lobules or their fragments contributing to the nodules (monolobular or multilobular) [2804], or distribution of the cirrhotic process (capsular, central, peripheral, periportal, or diffuse) [943]. Classification of cirrhosis according to these characteristics is of relatively little value, since these features are frequently only different stages of the same process, rather than different types of diseases.

In describing the cirrhotic process, the morphogenetic, etiologic, and functional viewpoints are

more satisfactory, all three of which are required for adequacy.

MORPHOGENETIC CLASSIFICATION. Various terms are in general use to designate the morphogenetic pathways leading to cirrhosis. The attempt has been made to differentiate Laennec's, or portal, cirrhosis from postnecrotic cirrhosis [1201], as reflecting differences in development of the cirrhotic process. On the basis of the morphogenetic pathways discussed previously, the differentiation of postnecrotic cirrhosis, septal cirrhosis, and periductular fibrosis, or pseudocirrhosis, is proposed (Fig. 120). The last group is usually designated as "biliary cirrhosis," but this term is avoided here because of its etiologic implications. Different pathways can frequently be recognized in the same liver. Septal cirrhosis may thus locally show broad bands characteristic of postnecrotic cirrhosis, while cirrhosis which is mainly postnecrotic may show all the characteristics of a diffuse septal cirrhosis in some parts of the liver. A terminal common pathway exists, in which diffuse nodularity of the liver is associated with diffuse septum formation, at least in part of the liver, either grossly or microscopically. The initial pathway can no longer be discerned, and the term "Laennec's

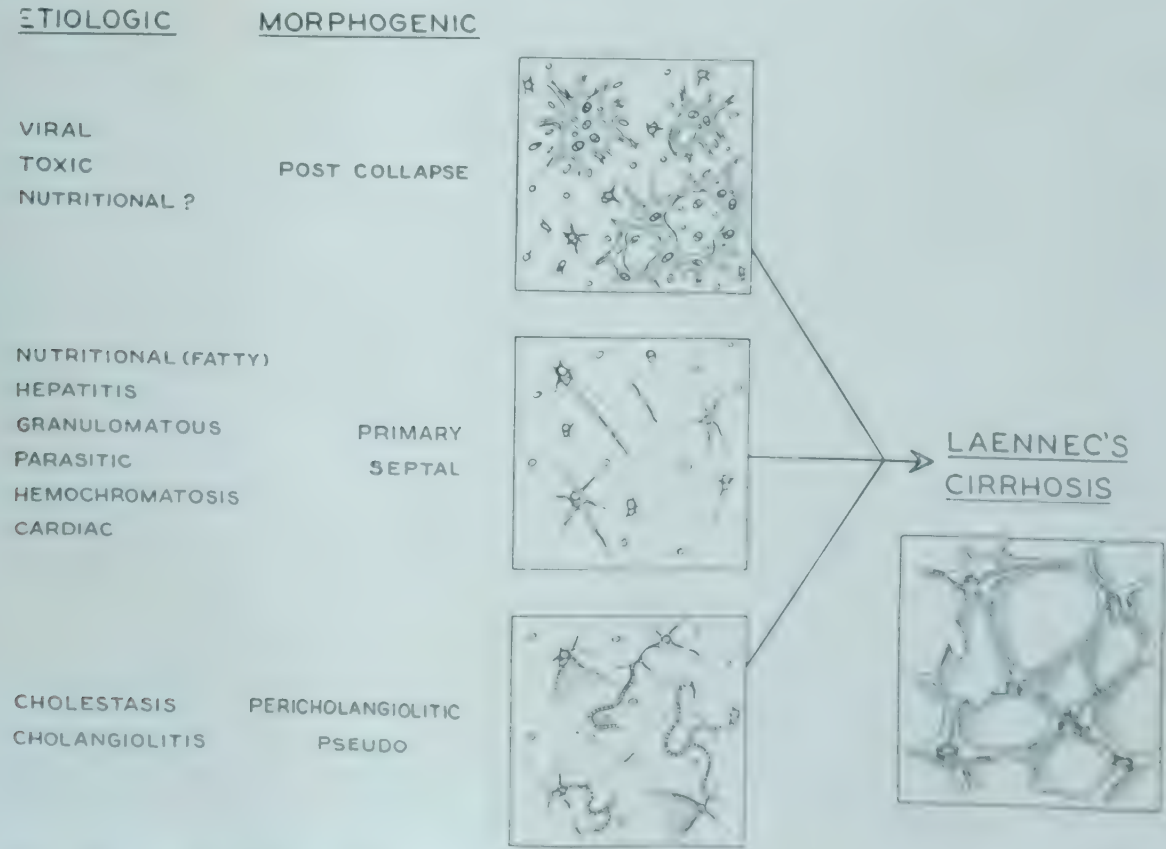


FIG. 120 Etiologic and morphogenetic factors as well as pathways of cirrhosis formation. (Popper, H., and Elias, H.: *Am.J.Path.* 31:405, 1955.)

rrhosis" is proposed for this terminal stage, in analogy to the use of the term "Bright's disease" renal disorders.

ETIOLOGIC CLASSIFICATION. An etiologic classification of cirrhosis is the most satisfactory from an academic and therapeutic standpoint, yet functional correlations are better with morphogenetic types, as well as with the stages to be discussed later. Moreover in many instances the etiologic factors are unknown or doubtful.

FUNCTIONAL CLASSIFICATION. This embodies the evaluation of criteria describing the degree of hepatocellular injury and the extent and rate of progression of the cirrhotic process (see Criteria for Clinical Evaluation of Cirrhosis, Chap. 52).

Human versus Animal Cirrhosis

Experimental production of cirrhosis in animals has been attempted for various reasons, among them the study of its morphogenesis, since the initial stages of many types of cirrhosis are unknown in human beings (see Chap. 50, Nutritional Hepatic Injury: I). The connective tissue

septums found in common forms of human cirrhosis have been assumed to originate from the portal tracts and have led to use of the term "portal cirrhosis." In experimental rat cirrhosis produced by low-protein-high-fat diets or by chronic carbon tetrachloride intoxication, the septums can be injected with charcoal gelatin from the hepatic vein but not from the portal vein [110, 1194, 1320, 1406]. This observation suggests that the septums originate from the central fields and that this type of experimental cirrhosis is central and not portal. The contrast between the more common types of human cirrhosis, especially Laennec's cirrhosis, and experimental cirrhosis is probably related to differences in blood supply [911]. The difference in initial morphogenesis is less significant in the advanced stage, because the septums reach the portal tracts in experimental cirrhosis and the central canals in human cirrhosis. In this sense both types of cirrhotoses are portocentral [2631]. Other types of experimental cirrhosis are more nearly analogous to cirrhosis in human beings (Table 12).

Table 12 Etiologic and Morphogenetic Differences between Human and Animal Cirrhosis

Type	Animal		Human	
	Etiology	Morphogenesis	Etiology	Morphogenesis
Fatty.....	High fat, low protein Carbon tetrachloride	Septums: 1. By "nonportal" fibrosis (fatty cysts) 2. In stress fissures by uneven regeneration	Malnutrition Alcohol	Septums: 1. After inflammation, membrane formation mainly portal 2. In stress fissures after uneven expansion due to regeneration, fat, necrosis 3. Around fat
Central septal....	Bromobenzene	Central membrane formation	Congestion	Central membrane formation
Portal septal....	Allyl formate	Periportal membrane formation	Granulomas Hemochromatosis, cholangitis Viral hepatitis	Periportal membrane formation
Postnecrotic....	Necrogenic diet	Collapse after massive necrosis		Collapse after massive necrosis
Biliary.....	Biliary obstruction Chronic ethionine	Periductular fibrosis	Biliary obstruction, "cholangiolitis"	Periductular fibrosis

REVIEW OF BASIC FUNCTIONAL ALTERATIONS

The main functional alterations in cirrhosis are (1) the effect upon hepatic-cell structure and function; (2) portal hypertension; (3) the effect upon the organism in general.

EFFECT UPON HEPATIC-CELL STRUCTURE AND FUNCTION. Hepatic-cell degeneration and necrosis are not essential features of the pathologic physiology of cirrhosis, although they may be caused by or associated with cirrhosis, or they may lead to it. Furthermore, the original cause of the cirrhosis, such as the hepatitis virus or toxic agents, may induce hepatic-cell injury. The bypass of blood from the lobular parenchyma as a result of intrahepatic portohepatic venous anastomoses and extrahepatic portocaval collaterals (see *Vascular Anastomoses, under Processes Common to All Types of Cirrhosis, Chap. 28*) produces a functionally inferior lobular or nodular parenchyma. This causes hepatocellular degeneration or necrosis or an increased tendency to them and is often expressed as reduced functional reserve of the liver.

PORTAL HYPERTENSION. The compression of hepatic vein branches by regenerative nodules, or possibly by fibrosis, interferes with the blood drainage from the liver. The increased contribution of arterial blood to the cirrhotic liver and the many anastomoses between the portal vein and hepatic artery branches increase the amount and pressure of the afferent blood flow. Both factors account for portal hypertension in cirrhosis, although the hepatic vein compression is probably more significant.

SYSTEMIC EFFECTS. The effects of cirrhosis upon the organism as a whole are the sequelae of he-

patic-cell degeneration, such as jaundice and hyperestrogenism; of portal hypertension, such as bleeding esophageal varices and hypersplenism; and of blood bypassing the parenchymal hepatic cells via intrahepatic portohepatic venous anastomoses or extrahepatic portocaval anastomoses, such as portosystemic encephalopathy [3044]. Sometimes the effects of these sequelae are combined, as in ascites, which results both from portal hypertension and from hepatic-cell degeneration, or hepatic coma, which results both from blood bypassing the parenchyma and from hepatic-cell degeneration.

In correlating these functional changes with the structural changes inherent in the distorted reconstruction characteristic of cirrhosis, the regenerative nodules and the vascular anastomoses appear to be the most important features. Since they are produced by the same processes, necrosis and septum formation, their extent is usually parallel in individual instances, although they develop independently of one another.

RELATION OF STRUCTURAL AND FUNCTIONAL ALTERATIONS

Appreciation of the relation between altered function and structure in cirrhosis was enhanced by liver biopsy [3467]. Several morphologic features in cirrhosis are reflected in clinical and laboratory alterations, viz., hepatocellular degeneration, fatty metamorphoses, regeneration, hepatic inflammation, and scarring.

Hepatocellular Degeneration. The degree of hepatocellular degeneration in cirrhosis varies from none to very severe. It may occur in any form of cirrhosis, and only the prolonged duration distinguishes the changes from the functional alterations

of acute hepatitis. Hepatic-cell degeneration is an important factor determining the acuity of the clinical phenomena. It is the feature best correlated statistically with clinical or biochemical evidence of progression of the cirrhotic process. In a series of hepatic tests studied, increased cephalin flocculation and thymol turbidity best reflected the degree of hepatic-cell damage [2651]. In many instances functional impairment is absent, so that the hepatic tests fail to reveal abnormalities, and only liver biopsy demonstrates the presence of cirrhosis [2760]. These latent cases of cirrhosis and the similarity of the functional changes in cirrhosis and acute hepatitis indicate the diagnostic superiority of liver biopsy over the hepatic tests. The alterations of hepatocellular function are also reflected in histochemical changes, especially of nucleic acids and enzymes [3241, 3287, 3615].

SERUM-PROTEIN ALTERATIONS. Reduction of serum albumin is a typical, although not specific, laboratory finding in cirrhosis. A good correlation is found between the serum-albumin level and the clinical course [2661]. Ascites is an important factor in the further reduction of the level. Reduction of serum albumin is caused either by increased breakdown or reduced formation [3203, 3382, 3438]. Reduced formation is more in keeping with the known formation of albumin by the liver and with the depletion of cytoplasmic basophilia characteristic of liver disease. The albumin reduction is correlated with the degree of hepatocellular degeneration, according to some groups of investigators [2651, 3554], although others deny this [2662]. In cirrhosis without hepatic-cell degeneration, serum-albumin level is normal [2763]. Many electrophoretic studies confirm the albumin reduction [1260, 2087, 2228, 2627, 3205] and indicate that the alpha globulin values are unchanged on the average, although the range of values is greater than in normal persons. Beta globulins are higher in cirrhosis as a rule, especially in patients with jaundice, but similar high levels are also seen in acute hepatitis. Serum-gamma globulin level is consistently elevated, reflecting the progress of the disease. It returns to normal with recovery [3205]. The gamma globulin elevation mirrors mesenchymal reaction and only indirectly hepatic-cell degeneration. A gamma₁ component appears in cirrhosis, more than in acute hepatitis [1073]. It is the fraction in which the antibodies are found. Fibrinogen is elevated in cirrhosis, in contrast to the situation in acute hepatitis [1073, 3335, 3593]. Changes

of the protein fractions are the basis for the flocculation tests. Since the total globulin level is usually elevated, the total protein is not often reduced.

BILE PIGMENT METABOLISM. Jaundice in cirrhosis is correlated with the degree of hepatocellular degeneration [2651, 2758], although the cause of jaundice is poorly understood. The increased urinary urobilinogen excretion found in cirrhosis is also an expression of hepatocellular degeneration.

LIPIDS. Of the many alterations in cirrhosis, the reduction of the cholesterol ester ratio is one of the most consistent [2195, 3256]. The plasma-vitamin A level is also very low [2641, 2696]. This decrease is greater than in acute hepatitis, especially when jaundice is present. Abnormal dark adaptation in cirrhosis is a reflection of altered intermediary metabolism of vitamin A [2532].

ENDOCRINE CHANGES. Many endocrine changes occur in cirrhosis which are a reflection of hepatic-cell degeneration. Such effects are not restricted to cirrhosis. They may occur in some instances of acute hepatitis; they are, however, much more common in cirrhosis, some to the point of being almost pathognomonic. These are particularly the signs of hyperestrogenism, such as spider nevi [191, 2535], palmar erythema [2719], gynecomastia [220, 2318], testicular atrophy [220, 2351, 2722], and nodular hyperplasia in the prostate [220, 2036, 3662] (see Effect of the Liver on Estrogens, Chap. 62).

Fatty Metamorphosis. Fatty metamorphosis is extremely severe in some types of cirrhosis and completely absent in others. Although it is of great importance in the establishment or maintenance of the cirrhotic process, it has little influence upon hepatic function (see Functional Manifestations, Chap. 26).

Regeneration. The regeneration found in cirrhosis has functional significance for several reasons. The growing regenerating nodule compresses the hepatic vein branches and thus is one of the main causes of portal hypertension [1724]. Regeneration restores many of the functions lost by degeneration of large parts of the liver. The association of regeneration with degeneration explains the often intact function of the cirrhotic liver even with far-advanced reconstruction [2760]. This interplay between degeneration and regeneration is the main reason for the often poor correlation between histologic findings and results of hepatic tests in cirrhosis [648]. Regeneration carried to

an extreme may lead to carcinoma formation, explaining the frequent coincidence of cirrhosis and hepatocellular carcinoma (see Relation of Carcinoma to Cirrhosis, under Human Primary Hepatic Carcinoma, Chap. 58). However, metastatic carcinoma is rare in a cirrhotic liver, probably because of the altered hepatic circulation (see Metastatic Tumors in the Liver, Chap. 59). Cirrhosis has little influence on other tumors [1353, 2556], although it is stated that tumors of the extrahepatic biliary passages and pancreas and also of the mouth, pharynx, larynx, and esophagus are slightly more commonly found in patients with cirrhosis [2556].

Inflammation. The inflammatory features in cirrhosis are response to hepatocellular degeneration to a greater extent than they are a primary mesenchymal reaction. In this sense the clinical and laboratory features in cirrhosis referable to inflammation are largely secondary to hepatic-cell damage. One laboratory finding seems to be particularly related to the inflammatory reaction, namely, elevation of serum-gamma globulin level, which is much higher in cirrhosis than in acute hepatocellular degeneration. Diagnostically this is an important criterion for the recognition of the transition of hepatitis into cirrhosis [2636]. Elevation of gamma globulin level in liver disease has been associated with hyperactivity and basophilia of the Kupffer cells and the mesenchymal cells in the portal tracts (see Globulin Formation, under Kupffer Cells as Part of the Reticuloendothelial System, Chap. 14). In cirrhosis a fair correlation exists between this activity and the gamma globulin elevation, especially as far as portal cellularity is concerned [3467], and the Kupffer cells and other mesenchymal cells are usually very rich in cytoplasmic basophilia [3287]. While some evidence indicates that the gamma globulin found normally is not derived from the liver, the excess serum gamma globulin in liver disease, especially cirrhosis, is probably formed in the liver (see Globulin Formation, Chap. 14). Several causes can be considered for the excess serum gamma globulin in cirrhosis, after compensatory elevation as a result of hypoalbuminemia or a response to a nonspecific infection has been excluded [3335]:

1. Extrahepatic formation by plasma cells has been claimed [288], but plasma cells are not necessarily increased in the bone marrow or spleen in cirrhosis.

2. Hypersplenism may play a role (see Hypersplenism, under Portal Hypertension, later in this chapter).

3. Elevation of gamma globulin level in cirrhosis may reflect antibody formation.

4. Elevated gamma globulin level may be a reaction of the hepatic mesenchyma or reticuloendothelial system to hepatic-cell breakdown products. Specific antibodies against hepatic-cell breakdown products have been reported [290, 1265] but more commonly in acute hepatitis than in cirrhosis. Quantitatively, the amount of antibody possibly formed is not enough to explain the gamma globulin elevation, and it is necessary to assume the existence of a "reaction globulin" (see Globulin Formation, under Functional Characteristics of Hepatic Sinusoids, Chap. 14) possibly stimulated by antibody formation. Many serum changes are characteristic for mesenchymal injury in general. Such changes are found in infectious disease, collagen diseases, physical and chemical injury, and even malignant tumors. In addition to elevation of gamma globulin these changes include an increase in fibrinogen, alpha globulin, mucoprotein, C-reactive protein, and nonspecific hyaluronidase inhibitor [895]. Of these, gamma globulin, fibrinogen, and hyaluronidase inhibitor are elevated in cirrhosis, whereas alpha globulin, C-reactive proteins, and mucoproteins are often reduced [1274]. In view of the decrease of mucoprotein, it appears that the gamma globulin elevation in liver disease differs from that in other mesenchymal injury.

Scarring. Scarring, an important feature in cirrhosis, reflects the development of the cirrhotic process, including the collapse of the framework, fibroblastic proliferation, compression of the hepatic vein branches, and the production of portohepatic venous anastomoses. The main functional result of these features is a disturbance of hepatic circulation, which in turn leads to both portal hypertension and hepatocellular degeneration.

Much evidence for disturbed hepatic circulation in cirrhosis is available. In experimental cirrhosis produced by carbon tetrachloride, abnormal angiograms are seen, in contrast to acute hepatic injury [47], in confirmation of extensive studies by postmortal injection techniques. The hepatic blood flow is reduced in cirrhosis, as evidenced by impaired extraction of Bromsulphalein and increased hepatic arteriovenous oxygen difference [372]. Since Bromsulphalein retention reflects

impaired hepatic blood flow to a great extent, the retention found in cirrhosis, sometimes in the absence of other functional changes, measures the disturbance in hepatic circulation better than any other test. The hepatic artery supplies a greater share of blood than normal [2397], confirming observations on injection preparations [805, 2126].

The disturbed circulation leads to centrolobular or centronodular necrosis (see Central Necrosis, under Necrosis, Chap. 22). That scarring produces disturbances of bile flow and may be responsible for jaundice is suggested by some morphologic alterations but can not be proved in biopsy studies [2651].

PORTAL HYPERTENSION

Portal hypertension results from (1) venous block within the portal tree or liver, (2) impaired outflow of blood, or (3) excessive splanchnic blood flow or excessive hepatic arterial blood flow or pressure, even with a normal venous bed. The discrepancy between the available venous bed and the blood flow can be located either in the liver itself (intrahepatic portal hypertension) or, in approximately 10 per cent of the cases, in the main stem of the portal vein (infrahepatic portal hypertension). Finally the portal circulation in the liver may be embarrassed by suprahepatic factors (suprahepatic portal hypertension) (Fig. 121). Cirrhosis as an intrahepatic cause is the most frequent etiologic factor of clinically significant portal hypertension. The portal venous pressure in portal hypertension is the result of the degree of obstruction to the portal circulation, the amount and pressure of blood entering the portal system, and the number and size of the spontaneous or surgical portosystemic collaterals.

Measurement

Elevation of the portal pressure has been directly determined in the portal vein tributaries, such as the mesenteric or splenic veins, at laparotomy. In dogs, progressive constriction of the portal veins by cellophane bands raises the pressure above 120 mm water, as compared with the normal of 30 to 110 mm [1517]. In rats or cats, the pressure has been raised to over 200 mm, in comparison with the normal 70 to 140 mm [2743]. At the time of laparotomy in patients with cirrhosis, the pressure varies from about 250

to 600 mm water [298, 3552], in contrast with the normal of less than 220 mm [209, 3570] and the normal inferior caval pressure of 130 mm. Indirect measurements obtained by measuring the pressure in superior abdominal collateral veins

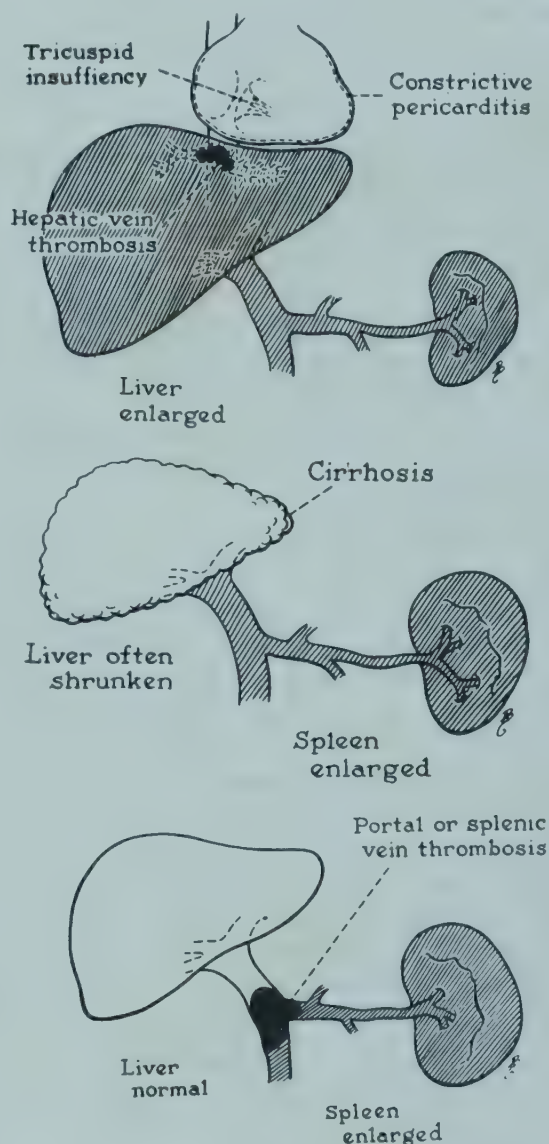


FIG. 121 Causes of portal hypertension and their effects on the size of the liver and spleen.

vary from 240 to 450 mm [733]. Indirect measurements in man can also be obtained by measuring intrasplenic or intrahepatic pressure through a fine needle [118], or by determining the pressure with catheters wedged into hepatic vein radicles [146, 2400, 2537]. Wedge pressures, supposedly measuring pressure in hepatic sinusoids, usually average 4 to 5 mm less than portal vein pressure. When portal hypertension is

produced by infrahepatic causes, the wedge pressure is almost always normal. Pressures necessary to occlude veins in the rectum [192] or the esophagus [2513] have also been used to estimate portal vein pressure.

Causes of Portal Hypertension

Portal hypertension is more severe the closer the absolute or relative obstruction is to the splanchnic system.

Suprahepatic Portal Hypertension. This results either from generalized arterial hypertension or heart failure with passive congestion. In both instances, the elevation of the portal pressure is relatively mild and does not lead to significant clinical symptoms except in the presence of tricuspid incompetency or constrictive pericarditis. The liver is usually large, and ascites is present (Fig. 121). Clinically, patients with suprahepatic portal hypertension present primarily the problem of cardiac failure.

Intrahepatic Portal Hypertension. The hepatic lesions producing portal hypertension are: all forms of cirrhosis, *hepar lobatum*, schistosomiasis even without cirrhosis owing to obstruction of the portal vein radicles, and occasionally hepatic tumors and fatty liver. Portal hypertension in cirrhosis was originally explained by fibrotic scar tissue compressing the portal vein tributaries and by the shrinkage and deformity of the portal vascular bed [2126]. The lack of correlation between the degree of fibrosis and portal hypertension and the observations of extensive anastomosis between the portal vein and hepatic vein tributaries [2631] militate against this original explanation. The recent studies of hepatic circulation in normal individuals and in cirrhosis indicate that two factors are of primary importance in the production of portal hypertension in cirrhosis. The first is compression of hepatic vein branches by regenerating nodules [1724]. Microradiographic techniques applied in France also suggest that the nodules interfere with emptying into the parenchyma of the portal vein branches in the septums. The second is increased arterial blood supply to the vascular network in the cirrhotic septums, supposedly due to interference with portal vein flow by scar tissue [2164]. This results in free presinusoidal communications between hepatic artery and portal vein branches in the vascular plexus developing in the septums. The hepatic arterial blood supply and hepatic arterial pressure in the portal vein branches combine with the

obstacles to the outflow from the hepatic parenchyma in raising the portal vein pressure (Fig. 122). In extreme instances a reversed blood flow in the portal vein branches toward systemic portocaval anastomoses must be assumed. Insufficiency of portal vein valves has little, if any, effect. Other factors, such as disturbed intralobular circulation owing to centrolobular necrosis and fibrosis, may also play a role. Portal hypertension mainly caused by hepatic necrosis, as in viral hepatitis or fatty metamorphosis, is usually transient, and its main sequela, esophageal varices, disappears with medical treatment. Transient portal hypertension also develops in infants in the recovery from severe nutritional deficiency [1216].

Other rarer forms of intrahepatic portal hypertension are caused by obstruction of hepatic vein branches. Portal hypertension from thrombosis of the major hepatic veins on an inflammatory basis, or Chiari's syndrome, resembles suprahepatic portal hypertension. Chronic rheumatic phlebitis of the hepatic veins may aggravate passive congestion from other rheumatic lesions and increase portal pressure [2798]. Visceral thrombophlebitis migrans may involve either the hepatic or portal veins [1149].

Intrahepatic portal hypertension is frequently associated with hepatocellular involvement, especially in cirrhosis, and occurs more often in older persons than the other forms.

Infrahepatic Portal Hypertension. PORTAL VEIN THROMBOSIS. Infrahepatic obstruction most commonly implies thrombosis of the portal vein (Fig. 121). Thrombosis starts at four main sites: (1) in tributaries of the intestinal vessels; (2) in the main stem of the portal vein; (3) in the splenic vein; (4) in hepatic branches of the portal vein. If it develops rapidly, the patient dies with symptoms of acute portal hypertension and hemorrhagic infarction of the small intestine. The lesion is usually associated with mesenteric thrombosis, which accounts for the acutely fatal splanchnic congestion, since ligation of the main stem of the portal vein in man does not produce this congestion (see *Interference with Portal Blood Flow*, Chap. 18). Portal vein thrombosis develops more frequently in men than in women. The clinical symptoms depend on the location and the degree of the obstruction. They are rapidly developing ascites without jaundice, hematemesis, vomiting, diarrhea with melena, abdominal pain, ileus, and death in coma.

In slowly developing portal vein thrombosis

extensive collaterals form and relieve the stasis. In addition, recanalization of the portal vein itself maintains some blood flow. Nevertheless, permanent and severe portal hypertension results [1801, 2189, 2449, 2733]. The thrombosis may produce shrinkage of the vein, resulting in a cordlike appearance with dilatation of the vein proximal to the narrowing, or the vein may become enlarged and recanalized with cavernomatous transformation. The degree of obstruction determines the prognosis and the clinical picture, which is dominated by epigastric pain, extensive collateral formation with hemorrhage from esophageal and gastric varices, splenomegaly with anemia and leukopenia, ascites, and rarely jaundice. The liver is usually small and atrophic and occasionally shows portal fibrosis. When the collateral circulation becomes insufficient, a severe, progressive portal hypertension may lead to rapid

death in a previously fairly well-compensated condition.

The causes of portal vein thrombosis are (1) spontaneous clotting following splenectomy, in polycythemia vera, or for unknown reasons, especially in the acute form; (2) hepatic cirrhosis, especially of the septal type; (3) inflammatory processes, such as appendicitis or cholecystitis in the portal tributaries with hematogenous spread, or by direct extension from pancreatitis or cholangitis, frequently via involvement of the regional portal lymph nodes; (4) compression of the vein by carcinoma, trauma, or by primary phlebosclerotic changes in the vessel wall comparable to arteriosclerosis [963]; (5) progression from splenic vein thrombosis. Splenic vein thrombosis alone does not embarrass the blood supply to the liver, but it causes portal hypertension with less pronounced esophageal varices. Splenic vein

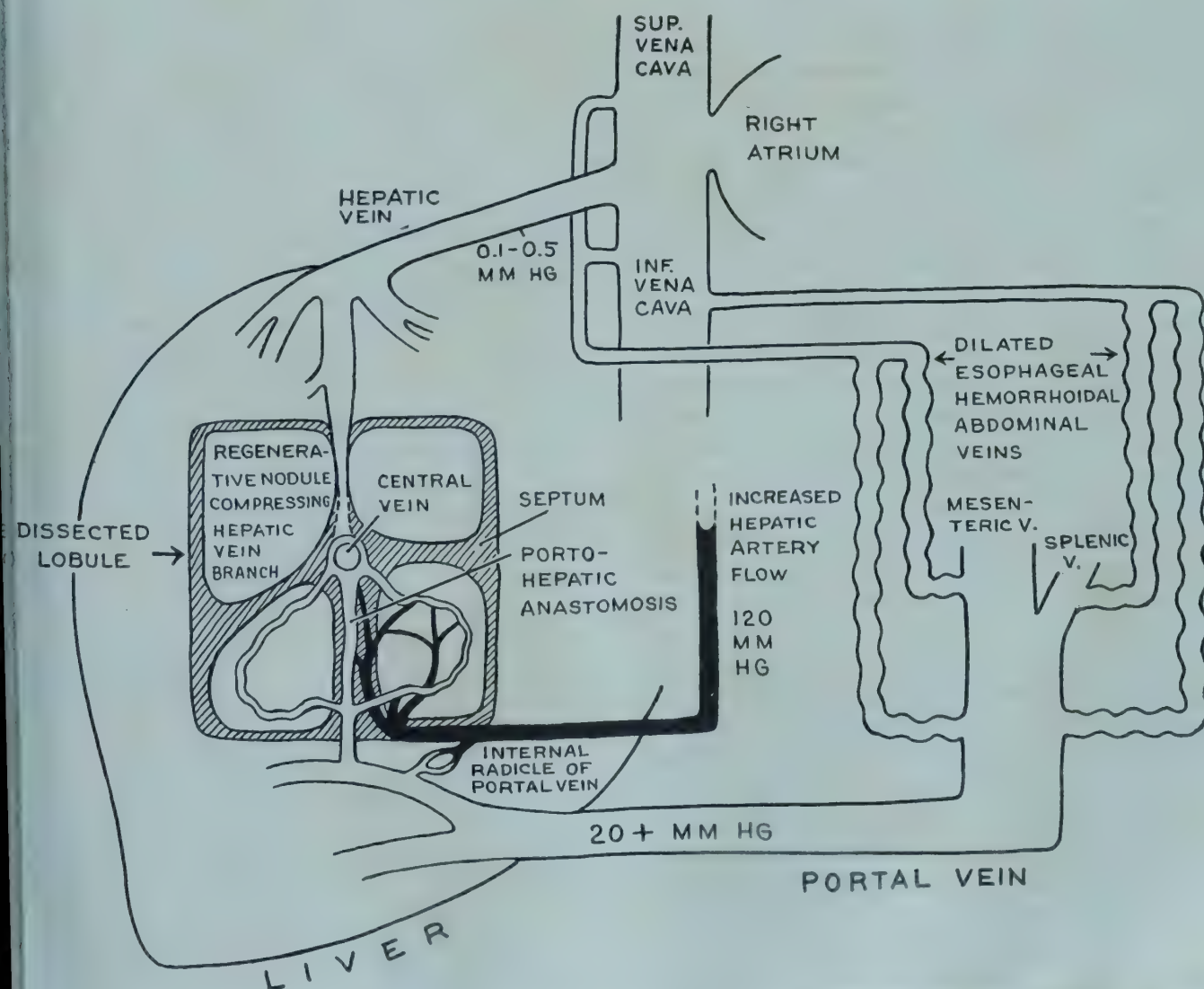


FIG. 122 Vascular communications and pressures in portal hypertension.

thrombosis may result from tumor invasion, inflammation, or granulomas.

CAVERNOMATOUS TRANSFORMATION OF THE PORTAL VEIN. This represents the replacement of the portal stem by a spongy mass. It may be a congenital malformation or allegedly a cavernous hemangioma but is usually the end result of portal vein thrombosis after recanalization [1159, 1801]. The lesion has been considered a sequel to postpartum umbilical infection [3109].

PORTAL VEIN COMPRESSION. Exceptionally, inflammatory masses or tumors, especially in the pancreas or enlarged lymph nodes, caused by Hodgkin's disease or sarcoidosis narrow or kink the portal vein and embarrass the lumen of the main stem without thrombosis [166, 3300].

CONGENITAL ANOMALIES OF THE PORTAL VEIN. Aplasia or obliteration of the portal vein which develops shortly after birth from progression of the normal atresia of the umbilical vein and ductus venosus [3552] and congenital strictures of the portal vein [2182, 3115] or hepatoportal arteriovenous fistulas [2169] cause portal hypertension.

CRUVEILHIER-BAUMGARTEN DISEASE. Persistence of the umbilical vein in postnatal life is the cause of Cruveilhier-Baumgarten disease, characterized by a communication between the portal vein and the systemic veins in the abdominal wall (caput medusae). This has been associated with congenital hypoplasia of the liver and its circulatory bed, thus explaining the portal hypertension [99]. This rare disease, without cirrhosis, must be differentiated from the Cruveilhier-Baumgarten syndrome (see under Venous Shunt Operations, later in this chapter), in which cirrhosis causes an unusually extensive dilatation of the paraumbilical veins.

PORTAL HYPERTENSION WITHOUT ANATOMIC CAUSE. Rarely, the clinical and anatomic picture of portal hypertension is noted without any demonstrable changes in the liver or portal vein itself except for some dilatation of the splenic and portal veins [943, 1723, 2317, 2840]. Abnormal microscopic arteriovenous anastomoses bypassing the capillary circulation in the spleen have been assumed [2557, 2722]. Grossly visible arteriovenous aneurysms in the spleen producing portal hypertension are very rare.

Experimental Production of Chronic Portal Hypertension

Portal hypertension has been experimentally produced by cirrhosis formation, for instance,

caused by chronic carbon tetrachloride administration or by the injection of silica into the portal venous system [1517, 2840, 3361]. Ascites usually develops after experimental cirrhosis, but dilated esophageal veins are not found as a rule, because an erect posture is apparently necessary for their development. In rats with silica fibrosis some esophageal varices, with dilatation of superficial abdominal and retroperitoneal veins but without ascites, have been reported [2840].

After supradiaphragmatic constriction of the inferior vena cava with a cellophane band, the portal pressure rises progressively to levels up to 270 mm water in rats, and passive congestion of the liver develops [1878, 2136, 3439]. Similar results are found if the inferior vena cava is ligated or greatly constricted [1731]. Dilatation of venous collaterals is noted. Hepatic vein ligation in dogs with portocaval shunts creates portal hypertension [692].

The third experimental method of producing portal hypertension is constriction of the portal vein simultaneously with ligation of the abdominal portion of the inferior vena cava. Ligation of the portal vein in dogs, in two stages, fails to produce portal hypertension, because collaterals rapidly form and keep the pressure normal. In rats, ligation of the portal vein does lead to portal hypertension [2743]. Shunts between the aorta and portal vein cause a rapid rise in portal pressure [617]. Transient portal hypertension can also be produced by various drugs. Epinephrine increases portal pressure as part of a generalized pressor effect, although not in cirrhosis [565], whereas histamine increases portal pressure while it lowers the systemic blood pressure [1701, 3462, 3570]. Severe portal hypertension with enlargement of the liver caused by constriction of the muscle bundles in the hepatic veins results in dogs from histamine or peptone administration or in anaphylactic shock. Food intake produces a temporary rise in the portal pressure, while acute hepatic damage and exercise do not influence it.

Sequelae of Portal Hypertension

Clinical Symptoms of Portal Hypertension. The enlargement of the spleen caused by portal hypertension leads to discomfort in the left upper quadrant of the abdomen. This discomfort may result either from stretching of the capsule or from the increased weight of the organ producing tension on its supporting structures. The congestion of the gastrointestinal tract which occurs

in portal hypertension may produce dyspepsia and meteorism. Finally, portal hypertension may be responsible for bleeding, either from esophageal varices or from hemorrhoids.

Effect upon Liver and Portal Vein. The liver itself is not affected by portal hypertension except for the passive congestion in suprahepatic portal hypertension. In infrahepatic portal hypertension, slight portal fibrosis results if the portal vein is obstructed. Portal hypertension leads to phleboscclerosis [2359] and sometimes even to thrombosis of the portal vein [2733].

Effect on Structure of Spleen. **GROSS APPEARANCE.** The effect of portal hypertension on the spleen is more severe the closer to its hilus the circulation is compromised (Fig. 121). Therefore, the changes are most outspoken in portal or splenic vein thrombosis [1723]. The lesion is called "fibrocongestive splenomegaly," in contrast to simple reticuloendothelial hyperplasia [2840]. The spleen is enlarged and firm. The capsule is thickened and sometimes covered by organized fibrin. It is generally slate colored. The cut surface is dark red and fleshlike. The follicles and trabeculae are irregularly thickened. The splenic vein is widened and tortuous, especially near the hilus.

The spleen often weighs over 500 gm, in contrast to the normal weight of 100 to 150 gm. In a study of 1,426 cases of cirrhosis, the spleen weighed less than 300 gm in 58 per cent, between 300 and 600 gm in 32.5 per cent, and over 600 grams in 8.5 per cent. In suprahepatic portal hypertension from cardiac failure, the spleen does not usually become so large as in cirrhosis and seldom exceeds 400 gm [2359, 3570]. The size of the spleen is not necessarily related to the degree of disturbance of hepatic blood flow in cirrhosis. Occasionally splenomegaly is associated with only minor hepatic changes (splenomegalic cirrhosis). In such instances, the splenic changes do not appear to be caused by hydromechanical factors alone, although they simulate the morphologic changes found with extensive hepatic involvement [2840, 3570].

HISTOLOGIC ALTERATIONS. The histologic changes in the spleen resulting from portal hypertension have been extensively investigated [713, 1802, 2158, 2359] and can be appreciated only with knowledge of the complex splenic circulation.

The Normal Spleen. The spleen consists of (1) trabecular extensions from the connective tissue capsule into the parenchyma; (2) the white pulp,

composed of lymph follicles, with or without germinative centers, arranged around terminal processes of the trabeculae like grapes; (3) the intervening red pulp, composed of sinuses surrounded by irregularly arranged meshes of pulp or Billroth cords [713, 1802, 1809, 2141]. The nature of the red pulp is debated. The cords contain a reticulum fiber framework, in contact with the fibers of the white pulp and anchored to the trabeculae. The framework supports a gel, in which the cellular elements are suspended. These are reticulum cells and histiocytes, some of which have very basophilic cytoplasm. Between these, plasma cells, lymphocytes, and a sprinkling of neutrophilic and eosinophilic segmented leukocytes are seen. The cellular lining of the capillary is not continuous. The lining cells are cytologically similar to the histiocytes or reticulum cells in the cords, and only rarely do they have the flat nuclei of endothelial cells. This morphologic similarity justifies the term "reticuloendothelial cells" for the entire group. No well-defined basement membrane of the sinuses is seen, and the reticulum fiber arrangement of the cords is irregular. This arrangement of the red pulp has been compared to a sponge in which cavities and septums change in function and position at various times. These changes occur because of splitting of pulp cords and fusion of the split part of one with parts of others.

Circulation through the Spleen. Arterial blood is supplied from the trabecular arteries and the sheathed arteries in the follicles through the penicillary arteries, which are the terminal branches in the pulp. The terminal arterial capillaries open with an ampullary portion into the pulp cord, so that blood outside an endothelially lined channel eventually trickles into the sinuses, i.e., an "open" circulation [2141]. Some assume that arteries deliver blood into the sinuses directly, at least in certain stages, i.e., a "closed" circulation [1809].

Portal Hypertension. Initially in portal hypertension, severe congestion leads to crowding of the sinuses and pulp cords by red cells, while the lumen of the sinuses is barely discernible. Subsequently, the pulp cords appear to break, and the pulp cells are widely dispersed. The follicles become surrounded by crescent-shaped accumulations of red cells, or blood lakes, which are not hemorrhages in view of the open circulation (Fig. 123, upper left). Eventually the red cells disappear from the pulp cords, and the cords condense

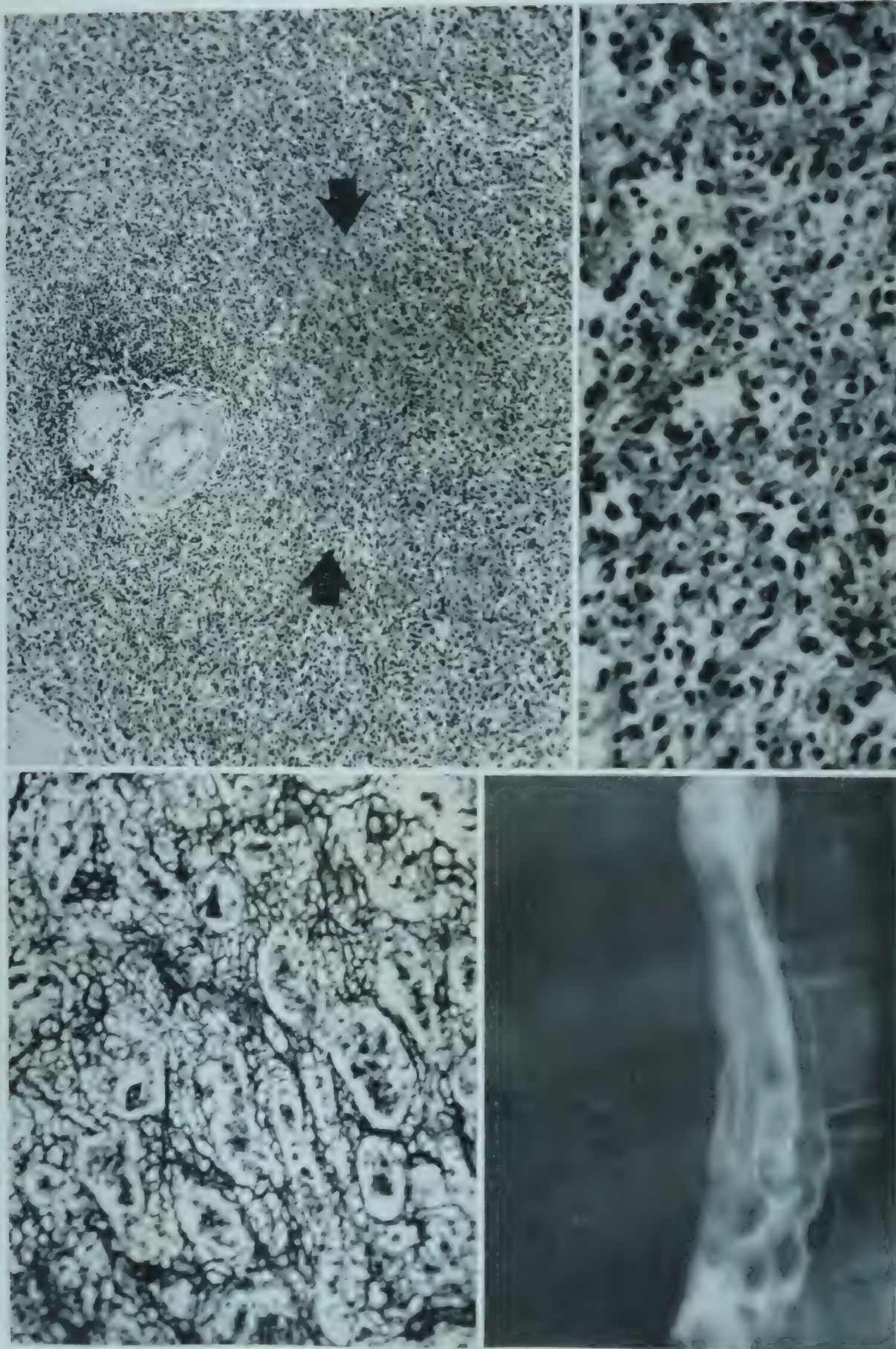


FIG. 123. Fibrocongestive splenomegaly. *Upper left*. Crescent-shaped hemorrhage (indicated by arrows) around small follicle and fibrosis of white pulp. H&E ($\times 76$). *Upper right*. Higher magnification of same, showing pseudogland formation owing to hyperplasia of endothelial cells lining the sinus and of reticulum cells. H&E ($\times 285$). *Lower left*. Newly formed reticulum fibers reinforcing the pulp cords and contributing to the glandlike appearance. Gamma silver impregnation ($\times 230$). *Lower right*. Esophageal varices demonstrated roentgenologically. (Courtesy of Dr. Gera G. Kopstein.)

with approximation of the cells. The sinuses become dilated, and the lining cells flatten. Five processes are responsible for further changes.

1. New formation of fibers in the pulp cords. These fibers form a basement membrane around the sinuses which eventually becomes collagenized (Fig. 123, lower left). This is associated with a gradual transformation of histiocytes into fibroblasts, appearance of elastic fibers, and decrease in white cells.

2. Swelling of the lining cells of the sinuses. These become large and homogeneous in appearance and show pallisading, thus simulating epithelial cells (Fig. 123, upper right). This has been called "pseudogland" formation or "fibroadenic" [943] and has been considered characteristic of Banti's syndrome.

3. Shrinkage and almost complete disappearance of the lymph follicles.

4. Fibrosis with blood pigment deposition in the crescent-shaped areas around the follicles, apparently resulting from organization of the blood lakes.

5. Reticulum cell hyperplasia. In many hepatic diseases, including cirrhosis, and independent of portal hypertension, reticuloendothelial proliferation with phagocytosis is present in the spleen. This is stimulated by hepatic-cell breakdown products or by the cause of the liver disease. The cellular response is greatest during the most acute hepatic-cell damage. Hemorrhage, from esophageal varices, for instance, accentuates reticuloendothelial cell proliferation.

The reticuloendothelial proliferation, together with new formation of fibers, as well as the distention of the sinuses with blood, accounts for the enlargement of the spleen. The reduction of white cells in the pulp is only relative. In severe fibrocongestive splenomegaly, many hematopoietic foci containing megakaryocytes appear [1802, 2359]. In the final stages, a closed circulation is present, and the pulp cords no longer serve as a filter [2359].

PATHOGENESIS. The mechanical effects of portal hypertension [2359] are the main cause of the splenic changes. However, a primary injury of the spleen, particularly in cirrhosis, is not entirely excluded. The extreme opinion is the concept of Banti, who postulated a primary splenic disease.

Effect on Function of the Spleen. Enlargement of the spleen from any cause, including portal hypertension, may lead to hypersplenism, imply-

ing an overactive spleen, with development of anemia, granulocytopenia, and thrombocytopenia.

BANTI'S SYNDROME. Banti's disease was described in 1894 as a condition characterized by anemia and splenomegaly, and was considered a primary splenic disorder. After 50 years of argument, Banti's disease is no longer considered a disease but is thought to be a syndrome which results from hypersplenism in portal hypertension [166, 943, 2359, 3329, 3552].

HYPERSPLENISM. An influence of the spleen on blood cells was postulated early, and the term "hypersplenism" was used in 1919 [943]. Now exaggerated splenic function is thought to reduce red cells, white cells, or thrombocytes, or any combination of these cells, in the circulation [713, 3636]. Two mechanisms have been considered: either increased destruction of corpuscular elements by phagocytosis in the spleen [3636], or production by the spleen of a humoral factor which depresses maturation or release of corpuscular elements in the hyperplastic bone marrow [624, 713].

The life span of the red cells is reduced in liver disease, especially cirrhosis [1633]. Passage of red cells through the spleen is said to prepare them for physiologic disintegration, and actually many of the red cells are hemolyzed in this organ. This process is increased in the hyperplastic spleen, accounting for the frequently increased hemolysis and hemosiderosis in liver diseases [1633]. In such instances, much iron deposition is demonstrable in the spleen.

Normal bone marrow function could compensate for the reduced life span of the erythrocytes, but since anemia develops, bone marrow maturation arrest has to be postulated. After splenectomy for relief of portal hypertension, leukopenia and thrombocytopenia are rapidly relieved, but not after splenic artery ligation or splenorenal anastomosis without splenectomy [2160].

Collateral Circulation. Increased portal pressure dilates collateral vessels between the portal and systemic circulation (Fig. 51) (see Hepatic Artery-Portal Vein Communications, under Processes Common to All Types of Cirrhosis, Chap. 28). The important collaterals are in three locations [2126]: (1) the junction of the glandular gastrointestinal epithelium and squamous epithelium, viz., the cardioesophageal border and the anus; (2) around remnants of fetal circulation in the round ligament continuing into the anterior

abdominal wall; (3) in the retroperitoneal space and in the diaphragm. The first two groups are clinically more important.

DILATATION OF THE JUNCTIONAL VEINS. Dilatation of junctional veins leads to varices in the lower part of the esophagus or hemorrhoids in the anorectal area. Esophageal varices are common in portal hypertension, regardless of its cause. They occur in liver diseases such as viral or amebic hepatitis without proved portal hypertension. In all types of cirrhosis and in carcinoma of the liver, varices in the lower third of the esophagus are rarely absent at autopsy [398]. The factors responsible for varix formation are not understood. The venous pressure in the varices is increased. The frequent association of varices with spider nevi in cirrhosis suggests that hyperestrogenism may be an etiologic factor [398]. Varices also occur in pregnancy. Not all esophageal varices result from portal hypertension, and occasionally an idiopathic form with dilated veins, sometimes in the upper half of the esophagus, is noted in older persons, possibly related to the negative intrathoracic pressure.

The veins in all layers of the esophagus are dilated in portal hypertension but they are most dilated in the submucous layer, because the soft areolar tissue permits expansion. The dilatation of the veins usually extends into the cardiac end of the stomach [2322]. As the submucous veins dilate, they bulge into the esophageal lumen and become recognizable roentgenologically [1774, 2911] (Fig. 123, lower right) or by esophagoscopy [398]. The dilated veins rupture as a result of pressure, trauma, or, more likely, peptic digestion of the mucosa [167, 3454]. Peptic ulceration, therefore, usually precedes rupture and mainly occurs in the esophagus. Hypoprothrombinemia and thrombocytopenia contribute to the duration and severity of the bleeding. Rupture of a varix is the most important cause of hematemesis and melena, although bleeding from a peptic ulcer (see Peptic Ulcer, under Influence of the Gastrointestinal Tract upon the Liver, Chap. 61) or diffusely from the gastrointestinal tract occurs in cirrhosis in jaundice [2804], and gastric varices may rupture [2804]. Hematemesis is a common clinical manifestation of cirrhosis, being reported in about 30 per cent of cases [2719]. It often determines the prognosis and is the cause of death in 10 [943] and 26 [2719] per cent of patients with cirrhosis.

Hemorrhoids are a clinically less significant

complication, being found in less than one-third of patients with cirrhosis [2719].

DILATATION OF ABDOMINAL VEINS. Dilatation of abdominal veins is not a dangerous complication but is of diagnostic value. It is not necessarily a sign of portal hypertension, since it also occurs after obstruction of the superior or inferior vena cava. The collateral veins seem to radiate from the umbilicus in approximately 25 per cent of patients with cirrhosis [943, 2719], although they are seldom extensive enough to justify the term "caput medusae." Dilated veins occur, usually with ascites [943], and disappear after paracentesis [1270]. They can be made more easily visible by infrared photography (Fig. 52). The extent of such veins demonstrated by this method parallels the degree of cirrhosis formation, as estimated by liver biopsy [1634]. Sometimes the veins produce conspicuous hum or thrill, for which a large number of theories has been presented [1996]. These venous hums are especially audible in the Cruveilhier-Baumgarten syndrome [99, 558, 1418] (see under Venous Shunt Operations, later in this chapter), in which the veins in the abdominal wall, particularly around the umbilicus, are excessively dilated. Portocaval-shunt operations in patients with the Cruveilhier-Baumgarten syndrome associated with cirrhosis cause disappearance of the veins and the hum [1627].

Treatment

The two main clinical manifestations of portal hypertension requiring therapy are esophageal hemorrhage and hypersplenism. Ascites, only in part a result of portal hypertension, is not greatly benefited by the therapeutic procedures applied in portal hypertension and often is a contraindication to their use. In previous years, splenectomy was encouraged for hypersplenism with anemia and leukopenia (Banti's syndrome). In liver disease, in contrast to splenic vein thrombosis, hypersplenism is seldom the main presenting symptom, and esophageal hemorrhage usually requires most of the therapeutic attention. Most patients requiring treatment of portal hypertension have the intrahepatic type, or cirrhosis, while only about 10 per cent have portal vein thrombosis, which is more common in young persons.

Esophageal varices have been obliterated by injection of sclerosing fluids [2322]. Recently emergency procedures have been developed for bleeding varices, such as topical application of ice water or thromboplastic agents, tamponade using various

multilumen tubes with balloons, such as the Sengstaken or Patton tube [2841, 3009], and ligation of varices [298, 689, 2016]. Ligation has been recommended to follow tamponade immediately. Definitive surgical techniques have been developed to eliminate the cause of bleeding by either portal decompression or removal of the bleeding collaterals.

Creation of Additional Collaterals. This was first performed by omentopexy, with fixation of the omentum to the abdominal wall—the Talma-Morison procedure. Collaterals form in the adhesions between the omentum and the abdominal wall and connect tributaries of the mesenteric veins with those of the inferior vena cava. Spontaneous as well as surgical omentopexy temporarily relieves portal hypertension but not sufficiently for any length of time [517]. Packing of the periesophageal area with gauze produces collaterals in the granulation tissues around the esophagus [2017].

Reduction of the Portal Bed. Removal of the spleen reduces the amount of blood entering the portal system [2557], and consequently the esophageal varices. The practical results of this procedure have been fairly satisfactory when hypersplenism is in the foreground. In cirrhosis, the results have not been so encouraging and do not compare with those of shunt operations [2557, 2840]. Hemorrhage has frequently recurred, and mortality is high [626]. Ligation of the splenic artery [243, 297] or the left gastric artery [243] is a substitute procedure if more extensive surgery is contraindicated. Splenectomy or ligation of the splenic artery, both of which often predispose to splenic vein thrombosis, destroys the opportunity for a splenorenal shunt [2016, 2219] and therefore should not be done if the surgeon is not prepared to construct a shunt. High gastric resection has also been recommended to reduce the portal bed [166, 3552].

Ligation of Hepatic Artery. The relative excess of arterial blood under high pressure over portal vein blood and the free communications between the hepatic artery and portal vein branches in the septums of the cirrhotic liver suggest hepatic artery ligation as a means of reducing portal hypertension. Such ligation has been performed distal to the origin of the gastroduodenal artery [242, 243, 2711] and has been combined with ligation of the splenic artery at its origin in the celiac axis [2654]. Ligation of the hepatic artery between the aorta and gastroduodenal artery has

also been recommended. The left gastric and splenic arteries should be ligated at the same time, with gradual occlusion of the hepatic artery [242]. The dependence of the liver on arterial blood speaks against hepatic artery ligation, although in cirrhosis it seems to be better tolerated [565]. Arterial blood coming through collaterals is probably required to maintain the liver after ligation, and the variable and unpredictable extent of these collaterals may be critical for the patient. The decreased blood flow following ligation may cause parenchymal anoxia and necrosis [3305], especially if portal vein branches are also occluded. No definite evidence has been obtained that hepatic artery ligation actually reduces portal venous pressure [146], but portal blood flow, important for regeneration, is not compromised by arterial ligation, as in the shunt operation [1283]. The results of arterial ligation thus far obtained are not entirely convincing, but remarkable effects in individual cases have been seen. The theoretical evaluation of the effect of hepatic artery ligation depends upon the importance attributed to the increase in arterial blood as against the compression of the hepatic veins in production of portal hypertension. Since the latter factor seems to be more important, the value of ligation is problematic. The procedure is justified if performed under local anesthesia in patients in whom shunt operations are contraindicated because of poor general condition, impaired hepatic function, or ascites.

Venous Shunt Operations. Portocaval or splenorenal shunts have been recommended for patients with portal hypertension [298, 565, 2016, 2840]. In portocaval shunts, the portal vein is anastomosed side to side to the inferior vena cava [298, 1628, 2840], or end to side with division of the portal vein, as in the original Eck fistula procedure. No agreement exists as to which is preferable. The end-to-side procedure avoids the continued effect of hepatic arterial pressure in the portal system, but it also makes possible the development of thrombosis in the intrahepatic portion of the portal vein. Splenorenal shunts involve removal of the spleen, after which the splenic vein is anastomosed to the left renal or spermatic vein. If an end-to-side anastomosis is performed, the left kidney may be spared [298, 2016, 2840]. If neither shunt is possible, mesenteric or umbilical veins have been used.

INDICATIONS. The most important indication for shunt surgery is esophageal hemorrhage or its im-

minent possibility. The operations give the best results in infrahepatic portal hypertension. In cirrhosis they are recommended if esophageal hemorrhage has occurred, although some investigators perform them prophylactically on mere demonstration of varices [2515]. Ascites, jaundice, and lowered hepatic reserve are usually considered contraindications because of the high operative mortality and because in cirrhosis the parenchymal blood supply may be already critically low [565, 2840]. Many surgeons do not operate if the sedimentation rate is high or if the serum albumin level is below 3.0 gm per 100 ml. Postoperative complications also appear to be increased if Bromsulphalein retention is high or cephalin flocculation is strongly positive. Preoperative control of ascites by resins has been suggested [1332], although in some instances ascites production ceased after a portocaval shunt was constructed.

SELECTION OF SHUNT. The selection of the shunt to be used is best made by either preoperative percutaneous, or intraoperative portal splenography, which visualizes the portal vein and its branches [2359, 2840] (see Hepatosplenoportography, Chap. 38). Portocaval shunt is generally preferable [1628] and should be performed if the portal vein is of normal size [2840]. This shunt is especially effective, since it relieves the pressure in the coronary vein, which is one of the main tributaries of the esophageal veins. If the spleen is very large, if the diameter of the portal vein is narrower than 1 cm, if excessive varicosities prevent adequate exposure, especially in the Cruveilhier-Baumgarten syndrome, or if the portal vein is thrombosed, a splenorenal shunt is preferable, although it often does not remain patent for more than 1 year. If the coronary vein is not dilated, splenectomy alone often suffices, although the construction of a splenorenal shunt is indicated to preserve the splenic vein. Sometimes a venous graft is required.

RESULTS OF SHUNT OPERATIONS. Shunt operations, if effective, reduce the portal vein from over 300 mm water [298] to normal levels or lower, approximating the mean 130 mm pressure in the inferior vena cava [2517]. The portal circulation time is also decreased [1166, 3466], and esophageal varices become less prominent or disappear [298]. The clinical results have been gratifying, particularly in the younger age group with portal vein obstruction without cirrhosis [298, 1667, 2016, 2017]. In cirrhosis with not too far

advanced liver damage, satisfying results have also been observed, although a stormy postoperative period is the rule. The results of hepatic tests after shunt operations vary; in half the patients the abnormalities increase while in the other half they decrease [298, 2517]. Bromsulphalein retention also varies in the postoperative period in cirrhosis [298]. The estimated hepatic blood is reduced after the shunt is established, but the extraction of oxygen and Bromsulphalein is augmented [375], forestalling the development of hepatic anoxia.

Since 85 per cent of cirrhotic patients with untreated esophageal varices die, shunt operations are indicated because they improve 75 per cent of the patients and less than 20 per cent die postoperatively [2840]. With improvements in techniques, mortality rates have dropped to less than 10 per cent. Even if the diversion of blood from the liver is adverse to hepatic function, the prevention of hemorrhage with shock and anemia is more important. Following shunt operations high-protein diets should not be used [1419].

Removal of Collateral-containing Areas. If a surgical shunt closes and esophageal bleeding recurs, or if the splenic vein has been obliterated by a preceding splenectomy, partial gastroesophagectomy has been recommended as a last resort [298, 2603], with a modification to prevent esophagitis [565].

ASCITES

Ascites is the accumulation of fluid in the peritoneal cavity that may occur as part of generalized edema or anasarca or may be present without it, especially in liver disease. In rare instances in cirrhosis associated with hemorrhagic tendencies ascites may be hemorrhagic [2199]. Ascites forms at a rate up to a liter a day [902]. By injecting various substances such as Bromsulphalein into the fluid, its volume can be measured [141]. The appreciation of the several factors that share in its pathogenesis is best based on experimental studies.

Examples of Experimentally Produced Ascites. **CHRONIC HEPATIC INJURY.** In cirrhosis experimentally produced by chronic carbon tetrachloride, ethionine, or allyl formate intoxication, and by high-fat-low-protein diets, for instance, ascites develops frequently but not regularly.

PASSIVE CONGESTION. Narrowing of the suprahepatic inferior vena cava causes progressive

ascites with sodium retention in most animals [2136, 3040]. Excess oral sodium intake increases the ascites, and sodium accumulates in the ascitic fluid [2925], which is rich in protein. Hepatic lymph, also rich in protein, is increased [2446, 2447]. Severe hypoproteinemia develops. The proteins in the ascitic fluid and the serum have similar electrophoretic patterns [1630, 2087, 2135, 2446, 3439].

CONSTRICTION OF ABDOMINAL VENA CAVA AND PORTAL VEIN. This constriction produces transient and less severe ascites, even when associated with plasmapheresis, than constriction of the suprahepatic vena cava [462, 3439]. Without plasmapheresis the ascites is slight or absent, and excessive administration of sodium has little effect [2925].

Pathogenesis of Ascites

In the explanation of ascites formation, Starling's law of edema formation still represents a good starting point. Several forces regulate extracellular fluid exchange. In all capillaries, including those of the peritoneum, the blood pressure in the arterial portion is about 30 mm Hg. The effective hydrostatic pressure exceeds the effective colloid osmotic pressure of the plasma proteins by approximately 7.0 mm Hg. This results in the filtration of almost protein-free plasma into the pericapillary space, in this instance the peritoneal cavity. In the venous limb of the capillary, the

blood pressure drops below the colloid osmotic pressure of the plasma proteins, the latter having risen owing to the hemoconcentration resulting from filtration. Almost the same amount of fluid is reabsorbed, the rest being removed by lymphatic drainage.

One condition for a proper balance of the extravascular circulation is the escape of relatively little protein through the arterial limb of the capillary. In experimental ascites produced by supradiaphragmatic constriction of the vena cava, labeled protein injected into the blood stream rapidly appears in the ascitic fluid [2138]. Similarly, proteins injected into the ascitic fluid are quickly found in the circulating blood, the exchange occurring at a rate of 4 per cent per hour [2950]. The rate of transfer of albumin across the peritoneal membrane is at least three times faster than that of globulin [2139]. In experimental ascites, tagged red cells are more rapidly transferred from the peritoneal cavity into the blood than under normal circumstances [2137]. The water itself circulates most rapidly, as measured by tritium-tagged water, 40 to 80 per cent leaving the abdominal cavity every hour [2667].

The protein content of the ascitic fluid of cirrhosis varies and is lower than in ascites from other causes. Mean levels of 0.5 per cent albumin and 0.7 per cent globulin have been reported [1630]. After repeated paracenteses the protein content rises [945]. The ascitic fluid pressure

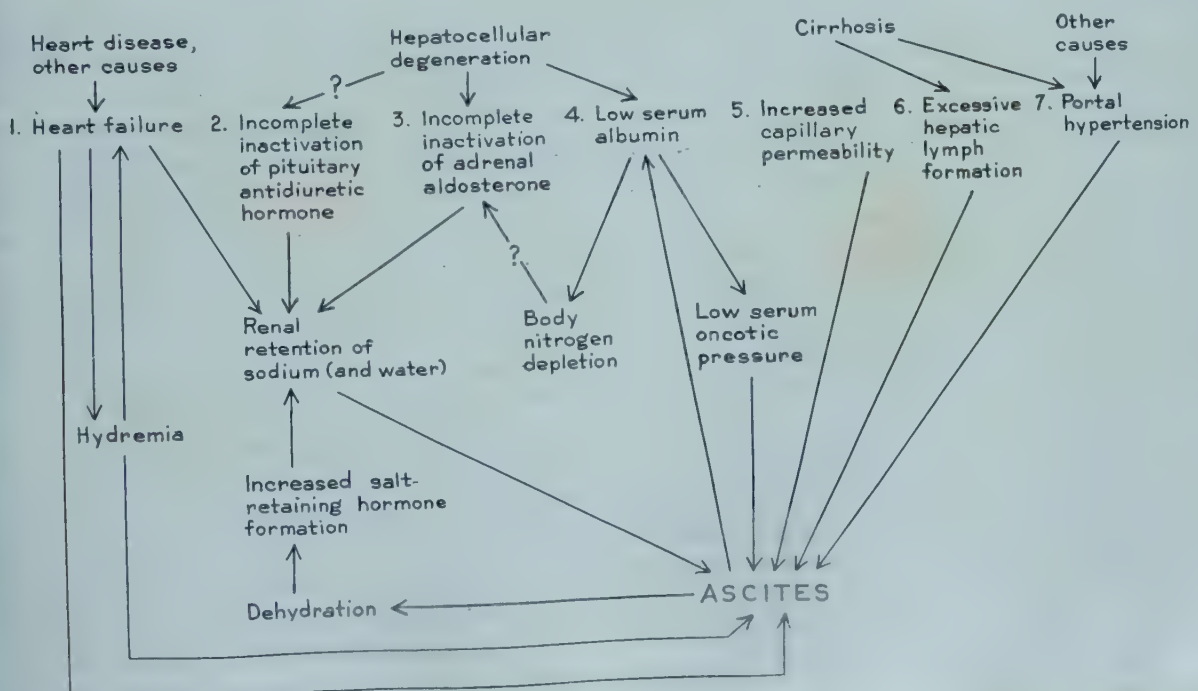


FIG. 124 Pathogenesis of ascites.

usually exceeds the peripheral venous pressure in cirrhosis, in contrast to other types of ascites [1630]. The presence of ascites also influences the venous pressure, especially in the abdominal veins [1979]. Several factors have to be considered in the pathogenesis of ascites (Fig. 124).

Portal Hypertension. Increased pressure in the portal vein raises the pressure in the venous limb of the capillaries of the peritoneum. This interferes with reabsorption, since the oncotic pressure no longer greatly exceeds the pressure in the venous limb of the capillary. Portal hypertension is not always associated with ascites, just as increased venous pressure does not necessarily cause edema. In the dog, obstruction of the portal vein does not produce ascites. In man, operative relief of portal hypertension by shunt operations does not necessarily alleviate ascites. In some patients with portal vein obstruction ascites is absent in the presence of portal hypertension, as evidenced by esophageal varices. In others, ascites disappears during sodium restriction and protein repletion without changes in portal pressure.

Anatomically, ascites formation in cirrhosis is associated with compression of the hepatic veins by regenerating nodules, and the number of hepatic vein branches demonstrable by injection techniques is decreased, while the portal vein branches appear to be normal or dilated [565, 2164].

In summary, portal hypertension is not the sole cause of ascites, but it probably is a most important contributing, localizing, and precipitating factor.

Hypoproteinemia. Reduction of the colloid osmotic pressure of the blood serum increases the difference between blood pressure and the water-binding power of the proteins, thus increasing filtration in the arterial limb of the capillary [2201]. It also reduces the blood volume and the difference between colloid osmotic pressure and blood pressure in the venous part, thus interfering with reabsorption. Albumin largely determines the colloid osmotic pressure, since it is a smaller molecule than globulin. Therefore, hypoalbuminemia, common in liver disease, is important in ascites formation. Although the colloid osmotic pressure can not be accurately predicted from the level of the protein fractions [100], the relation between ascites and low albumin levels in cirrhosis is well established [2661, 3467], while such a relation to the various globulins does not exist. Actually a vicious cycle develops as far as albumin is con-

cerned, in that loss of albumin into the ascitic fluid exaggerates hypoalbuminemia. Although hypoproteinemia, and especially hypoalbuminemia, is of great importance in ascites formation [286, 1483, 2661], the causal relations are not so simple as would be expected. Administration of albumin, for instance, does not stop ascites production but rather increases its rate of formation.

Sodium Retention. Retention of sodium with an increase in plasma volume is an important factor in ascites formation [2697, 3255]. Sodium is almost entirely absent from the urine [980, 2759], sweat, and saliva [904] of patients who form ascites. Almost all the sodium ingested enters the ascitic fluid, and the serum-sodium level is often low [904]. When ascites forms in cirrhosis after sodium chloride ingestion, the salt is retained isotonicity. When the salt administration is stopped, the accumulation of fluid ceases, and the urine increases by amounts comparable to the daily weight gain after salt ingestion. After paracentesis, water is retained in excess of salt [1114]. The sodium space of the body is increased in cirrhosis, although the exact reason for this is unknown [1168]. Water retention, usually secondary to sodium retention, may be the primary factor under some circumstances. For instance a water-retaining effect of excessive hepatic vasodepressor factor (VDM) has been claimed.

ALTERED RENAL FUNCTION. Disturbed renal sodium excretion has been considered an important factor of hepatic edema and ascites. The mechanism is still under investigation [237]. Two factors may be responsible; namely, reduced glomerular filtration and increased tubular reabsorption of sodium. Both factors may operate in cirrhosis [902]. Some evidence for decreased glomerular filtration has been reported [1959, 3078], but this does not seem to occur uniformly [946]. Increased tubular resorption of sodium is apparently more important.

EXCESS HORMONES. Excessive tubular resorption of sodium is assumed to result from an excess of either a pituitary antidiuretic hormone or a specific salt-retaining adrenal corticoid, aldosterone. This excess can be explained by two mechanisms possibly occurring simultaneously [731]. Faulty inactivation of either hormone by the damaged liver would result in salt and water retention with an increase in plasma volume. On the other hand, dehydration, owing to loss of fluid into the abdominal cavity during ascites formation and accelerated by paracentesis, may stimulate excessive

hormone formation [902, 2697, 3420], with further water and salt retention in the presence of a low plasma volume. Actually the plasma volume has been found high, low, or normal in cirrhosis with ascites, and both factors may be operating, producing a vicious cycle, but most investigators agree that faulty inactivation of hormones is the more important factor.

Aldosterone. Altered mineral corticoid formation or inactivation probably is the cause of increased tubular resorption of salt [296, 902]. The attempt to isolate a specific corticoid [553] has resulted in the finding of aldosterone, which is a potent salt-retaining hormone (see Desoxycorticosterone and Aldosterone, under Adrenal Gland, Chap. 62) and is increased in the urine during edema formation in cirrhosis [2088].

Pituitary Antidiuretic Hormone. Hepatic anti-diuresis because of excess pituitary hormone has been repeatedly claimed as a cause of ascites [520, 2522, 2701]. Augmentation of the antidiuretic activity of the blood in cirrhosis is not greater than in many other clinical conditions without water retention [520, 3188]. Furthermore, livers of normal and cirrhotic individuals inactivate antidiuretic hormone at the same rate in vitro [2290, 2291].

Increased Capillary Permeability. Protein escape beyond the amount which the lymphatic vessels can remove upsets the balance of Starling's law, in that the protein concentration in the pericapillary fluid has to be subtracted from the intracapillary colloid osmotic pressure in the venous limb. This results in reduced resorption of fluid and formation of ascites [943, 1654]. Causes of the disturbed permeability of the peritoneal capillaries are (1) anoxia, which contributes to the ascites in acute portal or mesenteric vein thrombosis, for instance; (2) protein depletion of the capillary wall [1689]; (3) inflammation, although the low concentration of protein in fluid militates against this. Changes in the volume of ascitic fluid or the amount of protein present do not alter the permeability [2950].

Lymphatic Changes. PERITONEAL LYMPHATIC OBSTRUCTION. The peritoneal cavity is drained by many lymphatic vessels. Their stomas on the peritoneal surface of the diaphragm have been demonstrated by dye absorption. Fibrin films, as well as fibrous adhesions, obstruct these lymphatic vessels, especially in cirrhosis, and thus facilitate ascites accumulation. Experimental obstruction of lymphatic vessels leads to ascites.

INCREASED HEPATIC LYMPH PRODUCTION. In experimental ascites, dilatation of the hepatic lymphatic vessels is noted. Escape of lymph from the liver surface into the peritoneal cavity is one of the main causes of ascites [340, 3436], but it is usually secondary to the other factors discussed.

Heart Failure. Heart failure is often present in patients with liver disease, either independently or caused by factors related to the liver disease, such as malnutrition, hydremia induced by sodium retention, or anemia as a result of bleeding varices.

Interrelation of Factors. In patients with liver diseases, more than one factor is active in the formation of ascites, the relative importance of each varying in individual patients and in different diseases (Fig. 124). In acute hepatic injury, especially if associated with jaundice, faulty disposal of the hormone influencing salt and water excretion appears to be the main cause of ascites. This is reflected in increased plasma volume during the disease and rapid diuresis upon its improvement. In chronic hepatitis without cirrhosis, sodium retention and hypoproteinemia are the most important factors. In cirrhosis, portal hypertension, hypoproteinemia, and sodium retention share in importance. When cirrhosis is associated with dehydration and low plasma volume, the diuresis on low-sodium diets is only gradual. In hepatic vein thrombosis, excess hepatic lymph formation and portal hypertension are probably the main factors. Therapeutic experiences indicate the interrelationship of the various factors. For instance, a high-protein diet, which restores the diminished body mass, induces sodium diuresis and reduces body water.

Sequelae of Ascites

CIRCULATORY EMBARRASSMENT. The accumulation of large amounts of fluids within the peritoneal cavity reduces the diastolic filling of the heart on one hand and increases the peripheral resistance in the splanchnic area on the other [2719]. Although these factors may partially neutralize each other, both ascites as well as its rapid removal by paracentesis disturb the circulatory efficiency of the heart.

EDEMA FORMATION. Pressure of ascitic fluid upon the inferior vena cava, especially in the recumbent position, increases the venous pressure in the lower extremities. Increased venous pressure in the kidneys increases sodium retention, which results in a vicious cycle. Pleural effusion is also commonly present [2719]. Inflammatory lesions,

such as coincidental tuberculosis or spread of a preexisting perihepatitis, are sometimes responsible for it. Abnormal abdominopleural communications have also been considered [3608].

RESPIRATORY EMBARRASSMENT. Elevation of the diaphragm compresses the lungs and thus decreases their vital capacity. This results in reduced oxygen saturation and cyanosis.

CHANGES IN BLOOD VOLUME. Plasma and blood volumes may be decreased because of the loss of fluid, or increased because of antidiuretic factors. Although the blood volume can be increased or reduced [1584], the tendency toward hypervolemia seems to predominate [1493, 2561].

DISTURBED RENAL FUNCTION. Disturbances of renal function are reflected in reversal of the normal diurnal rhythm, with an increase in glomerular filtration and effective renal plasma flow during the night [1657], apparently related to hormonal factors [1209].

HYPOPROTEINEMIA. Hypoproteinemia may be the result of ascites formation as well as a contributing factor in its formation. The predisposition to intercurrent infections, especially tuberculous peritonitis, is enhanced by deficiency of serum proteins [2719].

INTERFERENCE WITH INTESTINAL ABSORPTION. The increased intraabdominal pressure from ascites hampers the absorption of foodstuffs. This produces protein deficiency on a nutritional basis and the characteristic wasting of muscles, particularly of the extremities, seen in patients with chronic ascites.

ADHESIONS. Protein-rich ascitic fluid in chronic polyserositis produces fibrin films, which become organized over the peritoneal surfaces of the liver, spleen, intestine, and diaphragm and obstruct lymphatic drainage, producing another vicious cycle in ascites formation. Fibrous adhesions finally develop.

Therapy

PARACENTESIS. The mechanical removal of ascitic fluid is probably the most efficient procedure for relieving the cardiorespiratory consequences of ascites, and possibly also for improving renal excretion of sodium by reduction of the pressure upon the renal veins. Rapid removal of ascitic fluid may lead to untoward effects because of sudden lowering of peripheral resistance or of direct pressure on the heart. Sometimes acute cardiac dilatation or acute peripheral vascular collapse develops. The second untoward effect is a

rapid loss of serum sodium, enhanced by preceding administration of mercurial diuretics. This results in the low-sodium or salt-depletion syndrome [1530, 2426]. Another untoward effect is the rapid loss of serum protein, primarily albumin, into the ascitic fluid during its rapid new formation. Therefore, the peritoneal cavity should be emptied slowly, or small amounts should be removed at frequent intervals [243]. Paracentesis may also lead to contamination of the peritoneal cavity, facilitated by the hypoproteinemia. It should therefore be avoided whenever possible [2758]. Constant drainage of the peritoneal cavity by Cooney buttons [690] has been recommended, but its efficiency has not been established [2525].

MERCURIAL DIURETICS. The therapeutic benefits of mercurial diuretics are associated with increased urinary excretion of sodium [972, 1496], although opposition to their use has been voiced [1030].

SODIUM RESTRICTION. Rigid sodium restriction to approximately 17 mEq per day has been recommended as an efficient therapeutic procedure in controlling the formation of ascites [117, 903, 972, 1916, 2589, 2758]. The results are not always satisfactory, and symptoms of water intoxication or sodium depletion develop in some cases [902]. Ion exchange resins have also been recommended for the purpose of removal of dietary sodium [2361, 2811], but their usefulness is open to question.

HIGH-PROTEIN DIETS. The blood-protein level is raised either by a high-protein diet low in sodium [1691, 2534] or by parenteral administration of amino acids, albumin, or plasma. The high-protein diet may be supplemented by processed protein preparations derived from cereal products or by protein hydrolysates. The hydrolysates are prepared by acid or enzymatic digestion of various proteins. Methionine and tryptophane must be added to most of the hydrolysates, since their parent proteins are frequently deficient in them. Similar hydrolysates, properly purified, may be used intravenously. Oral administration of proteins in excess of 150 gm per day, including processed supplements, is soon met with aversion by the patient. The intravenous administration results finally in obliteration of veins, in addition to possible decrease in appetite. At present it is still a problem of patience and tenacity on the part of the patient and ingenuity on the part of the physician to vary the regime and to obtain palatable supplements.

Some patients do not tolerate a high nitrogen

take, and hepatic coma is occasionally precipitated.

PARENTERAL THERAPY. Whole blood, plasma, and salt-free albumin are administered as substitution therapy. Temporary improvement has been reported with daily administration of 25 to 75 gm

albumin over a prolonged period of time [972, 1881, 2663]. Combination with mercurial diuretics has been recommended [1421]. The original optimistic reports, however, have not been confirmed [973, 3506], and the metabolic fate of the albumin is unsettled.

In a discussion of the major syndromes of the hepatobiliary tract, the clinicopathological complexes which mainly involve the gallbladder but also concern the extrahepatic biliary tract must be discussed. They are important in both gastroenterology and surgery and are more fully discussed in the respective texts on these subjects. Discussion at this point centers around the relation of these complexes to hepatic function and structure. Three basic conditions are considered: biliary dyskinesia, cholelithiasis, and cholecystitis.

BILIARY DYSKINESIA

Dyskinesia, or dyssynergia, is a disorder of the motor function of the gallbladder and the extrahepatic biliary tract. It was first described by European schools, on the basis of findings resulting from cholecystography and duodenal aspiration [230, 2951], and was given a sound physiologic basis by the investigations of Ivy and his co-workers [1603], as well as by recent radiologic studies [2844]. No anatomic changes are found at operation to indicate the existence or nature of the phenomenon, and therefore it has frequently been denied. It is an incoordination of two usually synchronous processes, contraction of the gallbladder and relaxation of the sphincter of Oddi. Two types of dyskinesia, hyperkinetic and atonic, are described.

Hyperkinetic Dyskinesia

Hyperkinetic dyskinesia is either hypermotile or hypertonic. The hypermotile form is relatively quickly relieved [230] and is characterized by increased gallbladder motility. It responds to a fat meal stimulus by a very rapid emptying after a

short period of inhibition. In the hypertonic form, the gallbladder contracts in the presence of a spastic sphincter of Oddi. The gallbladder is distended, and mild colicky pain is present. A fat meal or administration of magnesium sulfate produces a long initial period of inhibition, which may or may not be followed by a prolonged period of flow of concentrated gallbladder bile [1680]. The obstacle to emptying was traced to a contracted gallbladder neck or cystic duct [2951], but the evidence for this is not convincing [1603], and it is more likely that the obstacle is caused by spastic contraction of the sphincter of Oddi [2544, 2844].

The pain reaction in dyskinesia is induced by distention of the ducts [229, 263, 1603, 3477]. In patients with spasm of the sphincter of Oddi and a biliary fistula, distention of the duct by a pressure of 30 cm water produces biliary tract pain. Similarly, the increase of bile secretion following bile salt administration produces pain if the ampulla of Vater is obstructed. Cholecystokinin, given when the sphincter of Oddi is contracted, also produces pain [1603], as does pilocarpine, which contracts the sphincter if given simultaneously with a fat meal. Colic after cholecystectomy, not referable to obvious disease of the liver or bile ducts, may also be initiated by sphincter of Oddi spasm [630]. The increased pressure in the biliary system may be short-lived or chronic. The corresponding symptoms are an acute attack of biliary colic with nausea, vomiting, and sharp pain, or chronic biliousness with distention, anorexia, and dull pain in the right upper quadrant [1605]. The acute attack often involves a previously irritated organ and is difficult to differentiate from gallbladder colic resulting from

cholelithiasis, which may also represent contraction of the gallbladder against an obstructed cystic or common duct. In this sense dyskinesia is synonymous with colic.

Causes of Hyperkinetic Dyskinesia. Excessive stimulation of the parasympathetic nerves produces sphincter of Oddi spasm in the presence of a contracted gallbladder, while with slight stimulation the sphincter remains relaxed and only the gallbladder contracts. Nervous stimulation with resulting incoordination of the gallbladder is probably the most common source of dyskinesia. The term "increased vagotonus" has been coined for this condition. It may be the result either of a generalized aberration of the autonomic nervous system caused, for instance, by emotional stress [130], or of an irritation of the intestinal parasympathetic system with overflow into the nerves of the biliary tract. This occurs with an irritable colon [1605] or in organic diseases of the gastrointestinal tract such as peptic ulcer, appendicitis, diverticulitis, or ulcerative colitis [1827]. In these conditions, the gallbladder may fail to be visualized during cholecystography. Chronic relapsing pancreatitis also alters the tone of the sphincter [1123]. Dyskinesia also results from inflammatory lesions in the biliary tract itself; such as acute or chronic cholecystitis, or from cholelithiasis. Allergic reactions have been accused of being responsible for dyskinesia. Finally, it is encountered in the presence of hepatic lesions. This last fact may produce differential diagnostic difficulties, which has led to the term "pseudocholelithiasis" [943]. Whether the hepatic disorder produces an imbalance of the autonomic nervous system, or whether irritation of the nerves in the gallbladder bed is responsible for the dyskinesia is not clear.

Sequelae. Sphincter of Oddi spasm has been said to produce jaundice [2544], as well as reflux from the biliary into the pancreatic ductal system, if the communication between the two ducts is sufficient (see Reflux between Pancreatic and Biliary Ductal System, under Interrelation between Gallbladder and Sphincter of Oddi, Chap. 16, and Relation between the Liver and Exocrine Pancreas, Chap. 61). Biliary dyskinesia is soon followed by anatomic changes in the biliary tract, mainly cholelithiasis and cholecystitis. The retention of bile for an abnormally long time in the gallbladder (biliary stasis) leads to increased concentration of the bile, followed by precipitation with subsequent stone formation. In dyskinesia, chemically irritating substances or bacteria may

remain for an unduly long time in the gallbladder, and cholecystitis subsequently develops.

Atonic Dyskinesia

Atonic dyskinesia is characterized by a reduced flow of bile during duodenal intubation. The gallbladder reflex is very slow, and the appearance of the concentrated gallbladder bile is delayed. This is usually associated with hypotony and dilatation of the biliary system and is apparently produced by distention of the colon or stimulation of its nerve supply.

This abnormality, in which the gallbladder is dilated and the sphincter of Oddi is relaxed, is probably induced by abnormal sympathetic tone. Hypomotility and dilatation of the gallbladder occur in liver diseases. They may persist for a considerable length of time in the convalescence after viral hepatitis [1677]. Atonic dyskinesia also accounts for the absence of a gallbladder reflex after fat-free diets, after bland diets, in dehydration, or during the fasting associated with surgery [2970].

Hypomotility causes stasis of bile in the gallbladder and predisposes to stone formation.

GALLSTONE FORMATION (CHOLELITHIASIS)

Gallstones result from precipitation of material normally dissolved in the bile in the gallbladder or the extrahepatic or intrahepatic bile ducts. Gallstones need not produce the clinical manifestations of gallbladder disease [17, 3364].

The bile is a supersaturated solution of bilirubin and cholesterol. The latter is kept in solution by the emulsifying effects of the bile acids [74] and fatty acids [3318]. Nevertheless, the solution is labile, and slight changes lead to precipitation of either cholesterol or bilirubin. In addition, admixture of calcium salts or protein causes fine precipitates and predisposes to stone formation. The physicochemical theory of gallstone formation, especially the role of an initial nucleus possibly formed from sloughed epithelium or wall particles as well as electrostatic and colloidal forces, found extensive discussion in the earlier literature [108]. In recent years alteration in chemical constitution found more emphasis [74]. The use of colloid chemical models has illustrated many physical phenomena characteristic of gallstones, such as faceting and the formation of Liesegang rings [1799].

Four processes operate in the formation of gallstones: (1) increased concentration of cholesterol or bilirubin in otherwise unaltered bile; (2) altered constitution of bile; (3) biliary stasis with increased concentration of solids in bile; (4) inflammation with exudation of calcium and protein into the bile from the altered surface of the gallbladder mucosa.

Shape and Constitution of Stones

The cause of stone formation is often reflected in the shape and constitution of the stones [108, 324].

Pure Stones. BILIRUBIN STONES. These consist of black, amorphous, friable, irregular masses or sand composed of bilirubin with a little admixture of cholesterol and calcium. Bilirubin, or earthy, stones are often found in the bile ducts, where they are brownish in color and rather soft and usually form casts of the ducts.

CHOLESTEROL STONES. These are firmer, yellowish-gray stones with a granular surface and a glittering cut surface, owing to the presence of radiating columns of cholesterol crystals. They are round or ovoid, they usually occur singly, and they may become quite large. Despite their size, these stones are radiolucent.

Mixed Stones. CHOLESTEROL-PIGMENT-CALCIUM STONES. These are the most common stones and are usually multiple, faceted, and pyramidal in shape. They may fill the gallbladder completely. They are brown and especially dark along the edges. These stones can be cut with only a moderate degree of difficulty. On the cut surface they usually have a dark center, sometimes with a cavity containing a pasty but glittering material. This is surrounded by layers containing glittering cholesterol and hard calcium.

PIGMENT-CALCIUM, OR MULBERRY, STONES. These stones are usually multiple and hard and have a granular surface.

Laminated Stones. Alternating layers of cholesterol, bilirubin, calcium carbonate, and mixtures of all three produce a very polymorphous appearance on the cut surface, reflecting the history of the formation of the stone and the multiple factors responsible. The initial nucleus, formed of cholesterol, may secondarily be dissolved, resulting in an empty space or slit, which may be replaced by subsequent deposition of calcium-bilirubin-cholesterol [108]. These stones often appear as large cylindrical solitary or double stones.

Calcium carbonate stones or calcified foreign

bodies, such as parasites, are rarely found. Bile acids are not part of the stones. —

Causes of Stone Formation

METABOLIC STONES. Although the constitution of bile does not necessarily reflect that of the plasma, some relation does exist, and hypercholesterolemia, as observed in pregnancy, obesity, diabetes, and hypothyroidism, is commonly associated with cholesterol or mixed stones. In some animals, stones develop on low-cholesterol diets and have no relation to the blood cholesterol [579]. Bilirubin stones resulting from hyperbilirubinemia do not occur in the obstructive or parenchymal types of jaundice, because under these circumstances the liver does not secrete enough bile pigment. In chronic hemolytic anemia, sickle cell anemia, or thalassemia with normal hepatic function, bilirubin stones occur frequently [3096, 3533].

ALTERATION OF BILE. Decreased concentration of bile acids or decrease of the bile acid/cholesterol ratio predisposes to precipitation [74]. This may be caused by inflammation of the gallbladder mucosa, permitting abnormal reabsorption of bile acids [see Inflammation (Cholecystitis), further on in this chapter]. The bile acid concentration in the bile may be reduced in liver diseases, facilitating the formation of stones [74]. However, the incidence of cholelithiasis in chronic liver disease, such as cirrhosis, is not increased [429]. Similarly the fatty acid concentration is important in keeping cholesterol in solution [812]. Changes in pH to the alkaline side, produced by stasis or hepatic disease, enhance stone formation [2018]. In inflammation or obstruction, the amount of calcium in the gallbladder increases, adding to any pre-existing stones. The type of stone which develops from these processes depends upon the nature of the alteration. Most stones are initially composed of cholesterol. The effect of diet in producing stones by altering the bile is not established.

BILIARY STASIS. Biliary stasis prolongs the stay of bile in the gallbladder. Bile salts diffuse out rapidly, and cholesterol, neutral fat, fatty acids, and bilirubin precipitate to form a pasty mass, which subsequently hardens. Biliary stasis also leads to an alteration of the pH, further predisposing to precipitation. Bile pigments, bacteria, or both break down the fatty acids, and they, too, diffuse out, leaving cholesterol with nothing to keep it in solution. Stasis is caused either by dyskinesia, with an incomplete gallbladder reflex, or by a reduction in the frequency of the gallbladder reflex.

The latter may result from a fat-free diet which does not stimulate the initiation of a gallbladder reflex.

Noncalculous obstruction of the cystic duct leads to precipitation of calcium carbonate, either as milk of calcium bile or as a layer around pre-existing stones [2584].

STONES FOLLOWING INFLAMMATION. Inflammation, especially from nonspecific cholecystitis [2018] and infections such as typhoid fever [355] or any other salmonella infection or even carcinoma of the gallbladder, predisposes to stone formation. Protein and calcium salts ooze from ulcers of the mucosa to act as nuclei for stone formation. Furthermore, bile acids are absorbed in inflammation, reducing the solubility of bile pigment and cholesterol. Moreover the inflamed gallbladder mucosa secretes increased amounts of mucinous material, which also enhances precipitation. The inflammatory stones are rich in calcium salts and are hard and radiopaque.

Interrelation of Factors in Stone Formation. In most patients a combination of factors explains the varied appearance and mixed composition of gallstones. Opposition has been raised on the basis of clinical experience and animal experiments to the roles of stasis and inflammation [324, 2018], although they frequently coexist with stones.

Cholelithiasis and Pregnancy. Pregnancy is commonly associated with the appearance of gallstones and is believed to explain the relatively higher incidence of stones in women [324]. Whether stones actually form during the pregnancy is not known, and some investigators deny any relation [2784]. The following factors play a role: (1) hypercholesteremia with increased bile cholesterol, which commonly occurs in the later stages of pregnancy [2572]; (2) increased vagotonus with delayed emptying [1150]; (3) distention of the gallbladder, possibly caused by mechanical pressure from the enlarged uterus [943].

Incidence of Gallstones. The high incidence of gallstones in women who are "fair, fat, and forty" can be explained by the hypercholesterolemia of obesity and by disturbances in emptying, although the validity of the dictum itself has been questioned.

The incidence of cholelithiasis is discussed in various texts [324, 1996]. It amounts to 10 per cent of the population in this country. It has been reported in children [2991, 3096] and is relatively common in young adults, although the incidence increases with age [2784]. It occurs more fre-

quently in white people than in Orientals or Negroes. Stones have been reported in 70 patients over seventy years of age [322]. An increased incidence is found in various diseases such as diabetes, pancreatitis, peptic ulcers, and pernicious anemia [1997]. Gallstones are said to form rather rapidly; the shortest time reported is between a few days and 3 months [324].

Choledocholithiasis. Most stones are found in the gallbladder (Fig. 125), but they also occur in the intrahepatic and extrahepatic biliary ducts (Fig. 125D).

In normal or dilated ducts or in pus-containing cavities, stones or amorphous gravel are found [176]. Ductal calculi usually are bilirubin-calcium stones, and their pathogenesis is similar to that of calculi in the gallbladder. The incidence of stones in the extrahepatic ducts without stones in the gallbladder probably does not exceed 5 per cent of all stones [1666]. Silent bile duct stones rarely occur, in contrast to gallbladder stones [17, 3364]. Hepatic calculi have been found in 10 to 20 per cent of cases with gallbladder stones [176, 262, 1479]. Stones in the common and hepatic ducts may be the result of movement of stones from the gallbladder through the cystic duct, or they may be formed there. Formation of duct stones usually follows precipitation of muddy or sandy material [3479] poor in calcium.

CHOLEDOCHOLITHIASIS AFTER CHOLECYSTECTOMY. Hepatic calculi are important causes of common duct obstruction after cholecystectomy and common duct exploration [262, 1479]. Calcium-rich "limey" bile [2098] precipitates in dilated ducts which have assumed depot functions after cholecystectomy.

CHOLEDOCHOLITHIASIS IN LIVER DAMAGE. Small grains of bile sand are the result of excessively concentrated bile in the presence of liver damage or cholangitis.

Hepatocellular injury as a result of infected biliary obstruction predisposes to choledocholithiasis. This is a further reason for stone formation after cholecystectomy if the diseased gallbladder was associated with infection or obstruction in the biliary tree.

THERAPEUTIC CONSIDERATIONS. Whether to explore the common duct in the presence of gallstones in the gallbladder is a question which is of major importance. Most stones in the common duct arrived there from the gallbladder as a result of migration or of spasmolytic treatment. They are hard and laminated, in contrast to the muddy

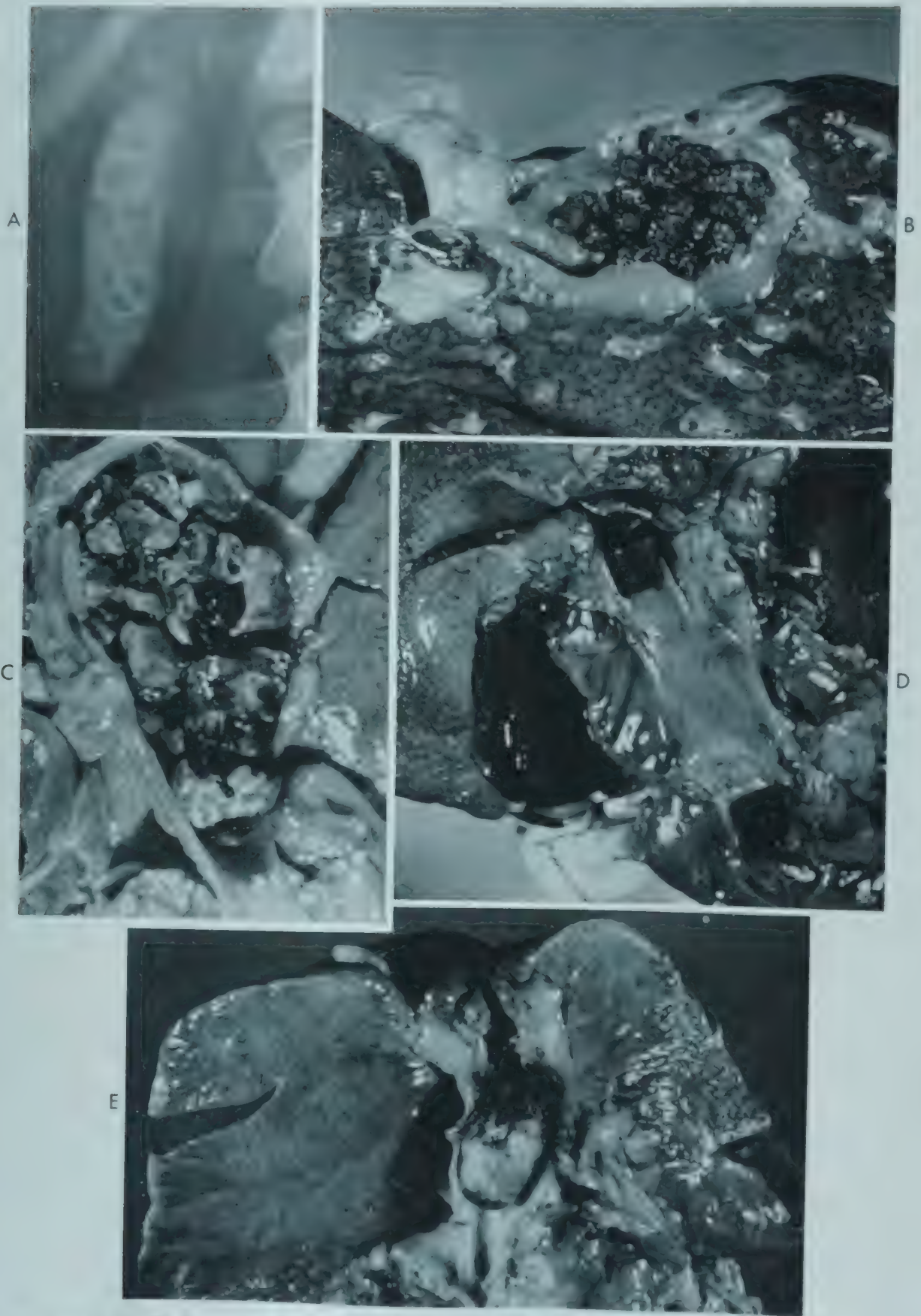


FIG. 125. A. Cholecystogram showing functioning gallbladder containing multiple faceted stones with faint rims of calcium. (Courtesy of Dr. Gera G. Kopstein.) B. Multiple mulberry stones in gallbladder with thickened wall. C. Gallbladder filled with mixed faceted stones. The lighter ones have calcium-containing shells. D. Two pigment stones, one in the cystic duct and the other at the lower end of a dilated common duct. E. Large solitary stone filling contracted gallbladder.

sand which originates in the duct. Of the many solutions tested for fragmentation or dissolution of gallstones at the time of surgery, chloroform has proved the best [265].

Sequelae of Gallstones

Dyskinesia. Irritation of the gallbladder mucosa results in subsequent nervous stimulation, with spasm of the sphincter of Oddi and dyskinesia. Together with obstruction of the cystic duct, this motor incoordination is the cause of the typical coliclike gallbladder attack. The location of the stones, as well as their size, is important. Impaction of the stone in the cystic duct of the neck of the gallbladder initially tends to produce dyskinesia and sphincter of Oddi spasm. Protracted impaction is not necessarily associated with dyskinesia or any noticeable clinical symptoms. The stones which do not produce symptoms can be classified as "silent" stones; the processes responsible for rendering such stones clinically active are not clearly recognized [3364].

Calculous Obstruction. Stones may obstruct the cystic and the hepatic, or common, ducts. In the first case, abolition of the gallbladder function results; in the latter two cases, jaundice is the result.

CYSTIC DUCT OBSTRUCTION. Stones may become impacted in the neck of the gallbladder or in the first portion of the cystic duct. The stones in the duct are smaller than those in the gallbladder neck. The obstructing stones are usually calcified. This obstruction leads to absorption of bile, including bile pigment and cholesterol, and filling of the gallbladder with a mucinous material, "hydrops of the gallbladder" (Fig. 81). The hydrops fluid, which is the product of the mucinous glands, must be differentiated from "white bile," which is a nonpigmented secretion of the liver. The gallbladder in hydrops is distended, firm, and tender. Sometimes the gallbladder is shrunken in cystic duct obstruction because of preceding inflammation and scarring. In the presence of infection during obstruction, empyema may develop. A stone impacted in the terminal portion of the cystic duct may cause obstructive jaundice. If both ducts unite at an extremely acute angle, the wall of the obstructed cystic duct protrudes into the lumen of the hepatic duct (Fig. 81).

OBSTRUCTION OF THE HEPATIC, OR COMMON, DUCT. Obstruction of the hepatic, or common, duct or the papilla of Vater by stones usually completely plugs the lumen, at least for a short period. The obstruction produces edema of the mucosa,

as well as a spasm, which soon subsides (Fig. 81). In addition, the duct dilates because of the obstruction, and within several hours or a few days, the obstruction becomes incomplete, with bile flowing around the stone. Intermittent complete obstruction may occur. Incomplete obstruction is often complicated by bacterial infection of the bile duct system. Only rarely does persistent complete obstruction result from stones impacted in the common duct or in the papilla, and in about half of the cases of choledocholithiasis, stones are found in the common duct without any jaundice. The gallbladder is usually not dilated because of the associated inflammation, in contrast to carcinomatous obstruction (Courvoisier's law, Simple Cholestasis, Chap. 47). Stones producing obstruction are often small and are usually derived from a reservoir of multiple-faceted stones in the gallbladder. Many obstructing stones are spontaneously dislodged and expelled into the duodenum, with spontaneous relief of the obstruction. Spasmolytic agents facilitate this process, as does anesthesia, which explains the absence of stones in the ducts at the time of surgery in some instances of calculous jaundice. Nevertheless, any instance in which a calculous jaundice has been relieved, either spontaneously or under medical therapy, is an indication for surgical intervention because of the probability of recurrence of the obstructive episode in view of the multiplicity of the small stones.

Inflammation (Cholecystitis). Cholecystitis may start from a decubital ulcer produced by calculi. Pressure ischemia of the mucosa leads to edema and predisposes to phlegmonous infiltration caused by secondary bacterial infection or granulomatous inflammation caused by biliary constituents. Scarring and contraction of the gallbladder are the end results. This course of events is usually initiated by large stones, generally solitary, which, because of their size, do not leave the gallbladder and which do not cause jaundice. The decubital ulcer may perforate, with expulsion of the stone into the peritoneal cavity or into an adherent portion of the intestine with formation of an internal biliary fistula. Calculous obstruction of the cystic duct also facilitates development of empyema.

Internal Biliary Fistula. Gallstones may leave the gallbladder and migrate through the biliary duct system, even passing through the narrow papilla of Vater. The relative size of the stones in comparison to the ducts is surprising. Sometimes stones the size of a walnut may pass through the natural passageways. The stone may also enter the

stomach, duodenum, or transverse colon through spontaneous fistulas from the gallbladder or the bile ducts, even from the terminal portion of the common duct near the papilla of Vater (Fig. 126C). Hemorrhage from the fistula may occur [1499]. Stones 2.5 cm in diameter and larger are delivered into the intestinal tract and may lead to obstruction of the intestinal lumen, or gallstone ileus, mainly in the terminal ileum [1050].

Carcinoma of Gallbladder. Statistically, carcinoma of the gallbladder is frequently associated with gallstones [1243]. In the presence of carcinoma, stones are found in between 80 to 90 per cent of cases [1653]. In carcinomas of the ducts, stones are found in only 35 per cent, and in carcinoma of the ampulla in 22 per cent of patients. Carcinoma of the gallbladder is found in 3.5 per cent of patients with gallstones. The question arises whether stones predispose to carcinoma formation by means of chronic irritation, or whether the inflammation associated with carcinoma leads to stone formation. Both possibilities must be considered, although the first is probably more important, since stones are infrequent in metastatic carcinoma to the gallbladder but occur in secondary deposits of carcinoma of the gallbladder in the liver [1710].

Bile possibly exerts a specific effect on carcinoma formation, and therefore hepatic dysfunction may play a role in the etiology of carcinoma. Bile acids are chemically related to carcinogenic substances and are possibly carcinogens themselves [645, 3195]. Furthermore, extracts of human bile are possibly carcinogenic [645].

Laboratory Diagnosis of Cholelithiasis

Aside from the various roentgenologic methods, which are the standard diagnostic procedures, duodenal drainage is helpful in the demonstration of crystals in the bile, a very common finding in the presence of choledocholithiasis. The biochemical changes resulting from the obstruction to the flow of bile in choledocholithiasis are discussed under cholestasis. Attacks of cholelithiasis without visible jaundice produce hyperbilirubinemia with a transient rise of prompt-reacting bilirubin within 24 hours [2909].

CHOLECYSTITIS

Cholecystitis is caused by the irritating effect of stones in 85 per cent of cases, although stones may also be the result of inflammation [794]. The

pathogenesis of noncalculous cholecystitis, especially of the chronic form, is a more difficult problem. The disagreement about the pathogenesis is reflected in different classifications. Both clinical significance and pathogenesis justify a division of inflammations of the gallbladder into acute, chronic, and recurrent cholecystitis.

Acute Cholecystitis. Acute cholecystitis can be divided into several varieties. The presence of a few leukocytes in the gallbladder wall is not necessarily abnormal.

CATARRHAL CHOLECYSTITIS. This condition, implied rather than proved, is the substrate of mild transient attacks owing to various factors, among which even allergy has been incriminated [60, 765]. It is rarely substantiated by biopsy or surgery.

ULCERATIVE OR PHLEGMONOUS CHOLECYSTITIS. This is a bacterially induced inflammation characterized by edema and diffuse infiltration of all layers of the gallbladder wall, predominantly the deeper ones, by segmented leukocytes. Occasionally it is not associated with ulceration of the superficial layers, but commonly it appears as a superficially spreading inflammation, diphtheritic in nature. The serosa is frequently covered by a fibrinous exudate. Many bacteria have been implicated, such as *Escherichia coli*, streptococci, and *Aerobacter aerogenes* [1207, 2029]. Salmonella, including *S. typhi*, is also frequently mentioned [355]. Gas-forming organisms are unusual offenders, producing an acute gaseous cholecystitis or pneumocholecystitis [1442]. Among these, *Bacillus welchii*, or *Clostridium perfringens*, is the most frequently encountered [1237].

CHEMICAL CHOLECYSTITIS. This is characterized by inflammation, again mainly localized in the deeper layers and not necessarily associated with ulceration of the mucosa. Severe hyperemia and edema with focal and diffuse infiltration with inflammatory cells are seen. Most of the cells are histiocytes and lymphocytes, with relatively few segmented leukocytes. The neutrophils are usually focally arranged [2143]. The inflammatory cells are found quite often around intramural lipid deposits, and granuloma formation appears very early [3551].

VASCULAR CHOLECYSTITIS. This is caused by interference with circulation and may take the form of focal infarcts. It is produced by polyarteritis nodosa, which typically involves the gallbladder, or necrotizing arteriolitis. Interference with the blood supply of the entire organ, resulting in

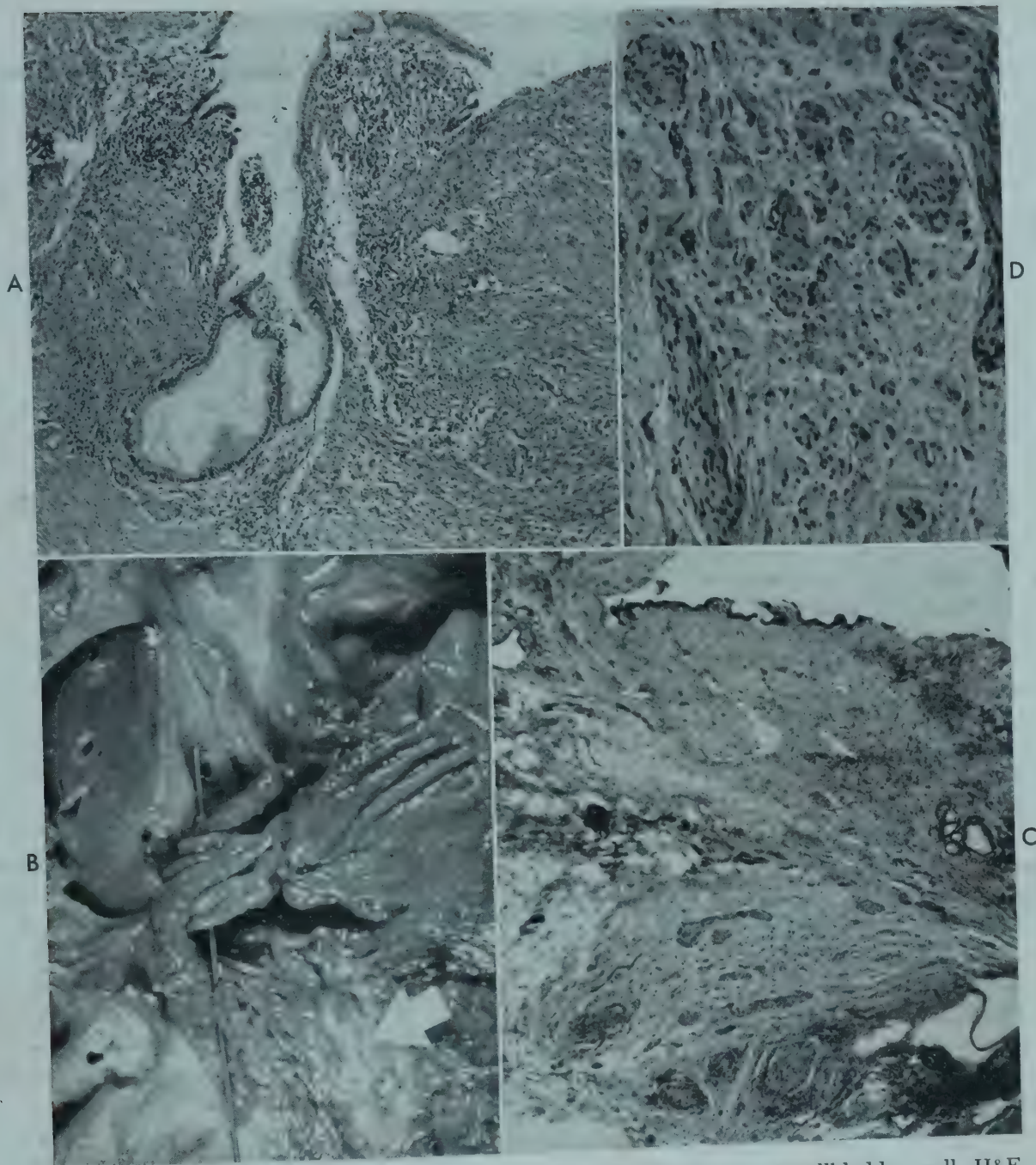


FIG. 126 A. Chronic cholecystitis with penetration of glands into the gallbladder wall. H&E ($\times 47$). B. Internal biliary fistula (containing probe) between duodenum and dilated proximal portion of common duct 2.0 cm above papilla of Vater (marked by white arrow). C. Painful proliferation of nerves in the subserous layer after cholecystotomy. H&E ($\times 27$). (Courtesy of Drs. T. Laipply and J. C. Sherrick.) D. Higher magnification of same, showing proliferating nerves. H&E ($\times 105$).

gangrenous inflammation, also belongs to this group [1189, 1357]. The nature of the interference can be obstruction of the cystic duct with pressure on the adjacent cystic vessels [597]. Torsion of the gallbladder also causes vascular interference.

Chronic Cholecystitis. Chronic cholecystitis is characterized by scarring in the gallbladder wall, with or without ulceration, associated with new

formation of elastic fibers [2774]. The gallbladder may be dilated or contracted, and its wall may be thick or thin. The lesion may be focal, or inflammatory cells may be found in all layers (Fig. 126A). The muscularis is often separated by thick layers of scar tissue, as well as by glands (Fig. 41). Outpouchings of the epithelial lining of the lumen may extend through all layers, forming sinuses (see Gallbladder, Chap. 15) which can

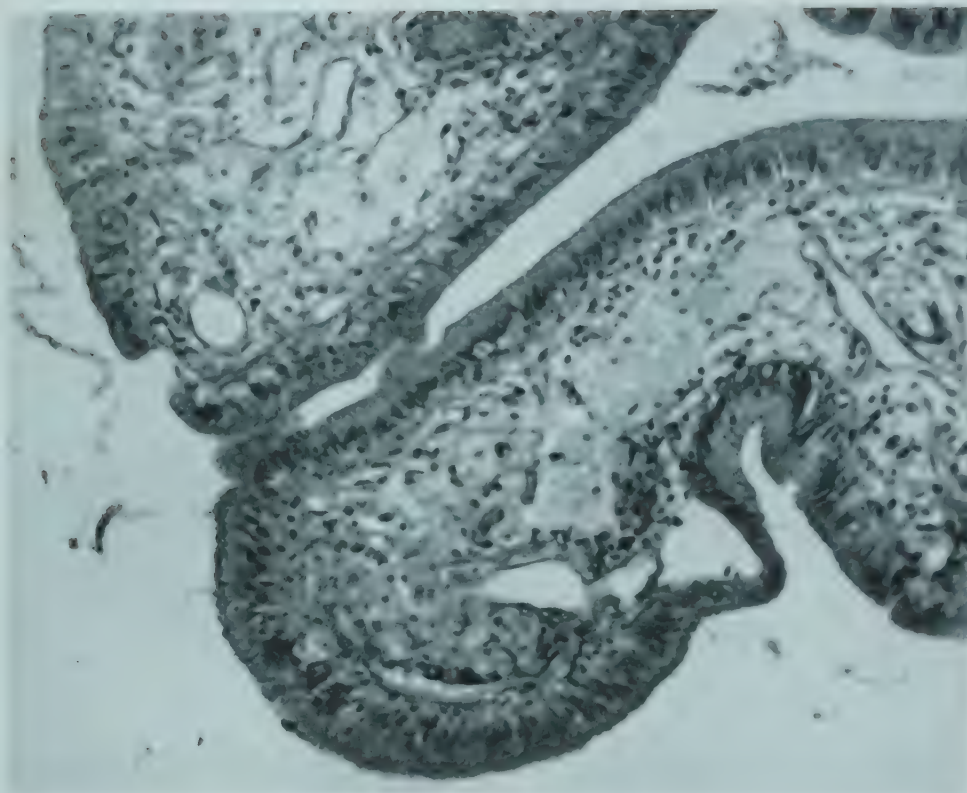


FIG. 127 Cholesterolosis of gallbladder. Foam cells in submucous layer. H&E ($\times 150$).

serve as a focus for persistence of the inflammation. The glands derived from these pseudodiverticula sometimes proliferate to form a glandular proliferating cholecystitis.

CALCULOUS CHOLECYSTITIS. Inflammation of the gallbladder by stones is the most common chronic form of cholecystitis.

CHRONIC CHEMICAL CHOLECYSTITIS. This develops from acute chemical cholecystitis and reveals conspicuous fibroplasia around the granulomas [3551]. Foam cells and even foreign-body giant cells are noted. The character of the exudate differs in this more frequent form from that in chronic bacterial cholecystitis [1135].

CHOLESTEROSIS, THE STRAWBERRY OR LIPOID GALLBLADDER. In this common lesion, cholesterol or cholesterol ester deposits are grossly visible as fine yellow patches in the mucosa [3551, 3648]. These may form polypoid papilloma-like structures and are precursors of stones. Histologically, in the early stages, small groups of histiocytes with foamy cytoplasm containing doubly refractile lipid bodies are found in the mucosa (Fig. 127). This condition may also be seen in severely inflamed gallbladders [3649]. The lesion results from abnormal resorption of cholesterol. The process is considered the precursor of chemical cholecystitis owing to lipid absorption.

CYSTIC DUCT OBSTRUCTION. Inflammatory obstruction of the cystic duct results from thickening of the wall, kinking, adhesions, previous stones, tumors, and enlarged regional lymph nodes.

EMPHYEMA. Empyema of the gallbladder, associated with obstruction of the cystic duct, occurs in two forms. In the less common one, hematogenously disseminated bacteria accumulate in the lumen of an obstructed gallbladder. In the more frequent form, emulsified small cholesterol droplets mixed with mucus give a puslike appearance, although only few leukocytes may be found. The clinical manifestations in the two instances are not so different as one would expect.

CALCIFIED CHOLECYSTITIS. Rarely, the gallbladder wall is transformed into a sac of partially calcified scar tissue.

CONTRACTED GALLBLADDER. This represents the end result of various lesions, among which stones are the most important. The wall of the gallbladder is severely scarred, its bed is thickened, and the function of the organ is abolished.

Recurrent Cholecystitis. In acute exacerbations of chronic cholecystitis, acute ulcerative and phlegmonous processes complicate the underlying chronic disease. Diverticula, occasionally containing intramural stones, serve as foci of further exacerbations.

Incidence

The incidence of cholecystitis in all its forms varies, depending upon the criteria applied. The literature records great discrepancies. The incidence at the autopsy table is higher than clinical manifestations would lead one to suspect. It is usually found in the presence of stones. Of the acute forms, chemical cholecystitis seems to be the most common. Chronic cholecystitis is seen frequently in autopsy material, but it is rarely the basis of clinical symptoms.

Pathogenesis

The etiology is readily apparent in calculous or vascular cholecystitis. Much argument exists regarding the relative importance of stasis, bacteria, and chemical irritation in noncalculous cholecystitis.

Stasis. Stasis of gallbladder contents has been considered as a cause of inflammation. Stasis results from either hypertonic or hypotonic dyskinesia, or from complete cystic duct obstruction. Prolonged experimental complete cystic duct obstruction [3648] or incomplete obstruction [625] of the cystic duct produces cholecystitis. Replacement of the bile by saline solutions in complete obstructions prevents cholecystitis [3648]. This indicates that the chief role of stasis in producing inflammation is to keep injurious bile in prolonged contact with the mucosa. The injurious agent in the bile is either bacterial or chemical.

Bacteria. A bacterial etiology of cholecystitis appears the most obvious. Many bacteria have been cultured from gallbladders in acute as well as chronic cholecystitis [74, 1207]. Lymph nodes draining the gallbladder have been cultured to avoid the bactericidal effect of the bile. However, the results of bacterial cultures are not well correlated with the pathologic changes of the gallbladder. In both severe acute cholecystitis and in chronic cholecystitis, cultures of the bile as well as of the gallbladder wall were negative [324].

ROUTES OF INVASION. Cholecystitis has been experimentally produced by inoculation of the gallbladder wall with bacteria, although not in all instances. Ascending infection of the bile from the duodenum or descending infection from the liver is unlikely under normal circumstances because of the bactericidal properties of bile. At bile acid concentrations of at least 70 per cent of that found in normal bile, no bacterial growth occurs. Under

abnormal circumstances, bacterial infection is common, particularly if ulcerations are present or if the gallbladder bile is altered and the absorption of bile acids through the inflamed mucosa is increased. Hematogenous infection is improbable, and in bacteremia, involvement of the gallbladder wall is uncommon, exception in typhoid cholecystitis. Lymphatic infection from the liver has been claimed on the basis of inflammatory changes in the liver, but similar changes are noted in many conditions, especially in the adult liver [2626], without any relation to cholecystitis [2450].

Factors other than bacteria are primarily responsible in the majority of cases of acute or chronic cholecystitis. Secondary bacterial infection, if present, results from settling of bacteria on a damaged gallbladder mucosa during stasis [1135, 3551].

Chemical Irritation. Two chemical irritants are to be considered. One is regurgitation of pancreatic juice into the bile, with consequent digestion of the gallbladder wall [283, 1135, 1504, 3643]. The frequent finding of amylase in the bile supports this assumption [2653], although cholecystitis is not necessarily associated with a common channel between the common duct and the pancreatic duct. Furthermore, the absence of severe digestion in the common duct speaks against this as a frequent cause of cholecystitis. Therefore, more emphasis is given to the constituents of the bile, particularly bile acids or cholesterol. Bile itself may be the offender, owing to its local toxic action [3648]. A transition from cholesterolosis, with cholesterol in the gallbladder mucosa, to chronic cholecystitis with lipid granulomas in the wall has been clearly shown [3551, 3648]. Moreover, inspissated biliary material in the pseudodiverticula maintains the inflammation. Whether cholesterol in the gallbladder wall is the result of resorption or excretion is not established. Some investigations incriminate the bile acids [1135], especially if they are abnormal owing to hepatic damage. Even if bile or pancreatic juice is the toxic agent, stasis with subsequent prolonged contact is necessary.

Diagnosis

The diagnosis of cholecystitis is usually made on clinical grounds and is based on the character of the pain and the typical right upper quadrant tenderness often associated with a palpable gallbladder. In the laboratory diagnosis of cholecystitis, the inflammatory character is sometimes

reflected in a high white blood cell count [1475]. Duodenal drainage is of assistance in indicating cystic duct obstruction by complete absence of the concentrated B bile of the gallbladder. Microscopic examination shows increased numbers of segmented leukocytes, while bacteriologic studies may or may not demonstrate bacteria. The results of the hepatic tests, especially Bromsulphalein retention [443], are frequently abnormal, especially in the acute form [2640]. The diseased gallbladder usually cannot be visualized by cholecystography.

Sequelae

The main consequences of cholecystitis are irritation of the ductal musculature with resulting dyskinesia and sphincter of Oddi spasm and cholelithiasis, as well as spread of the inflammation. The other sequelae are those typical of inflammation of a hollow viscus, such as perforation with bile peritonitis, internal biliary fistulas, pericholecystitis with subsequent pericholecystic adhesions, pericholecystic and subphrenic abscesses, and erosion of the cystic artery with hemorrhage [1996]. Perforation occurs in 6 to 12 per cent of cases of acute cholecystitis, about one-fourth of which terminate fatally [794, 2600]. The gallbladder perforates into the peritoneal cavity in one-third of all perforations, into adjacent hollow viscera in about 20 per cent, and in an encapsulated fashion, forming a pericholecystic abscess, in the remainder. Spread of infection to the liver, either by way of the portal vein with

or without pylephlebitis or by the lymphatic vessels through the gallbladder bed and ductal spread, leads to abscess formation or at least to toxic damage of the liver. Cholecystitis in children frequently causes hydrops of the gallbladder [1277, 1930].

INTERDEPENDENCE OF GALLBLADDER SYNDROMES

The various gallbladder syndromes are interdependent, in that any one of them can produce the others. This relationship is the cause of the vicious cycle characteristic of gallbladder disease (Fig. 128). The fourth main disease, carcinoma of the gallbladder, is also associated with the syndromes. As already stated, however, it is not established whether stones cause carcinoma via inflammation or whether carcinoma facilitates stone formation via inflammation.

The interdependence of the syndromes with its associated vicious cycle is also exemplified by some of the symptoms which occur following cholecystectomy.

Postcholecystectomy Syndrome. The postcholecystectomy syndrome is in part the result of avoidable or unavoidable errors or mishaps during surgery, such as ligation of the hepatic artery or common duct, laceration of the common duct with stricture formation, or stones left in the common duct. Erroneous preoperative diagnoses account for some of the instances seen.

Postoperative mechanical factors such as ad-

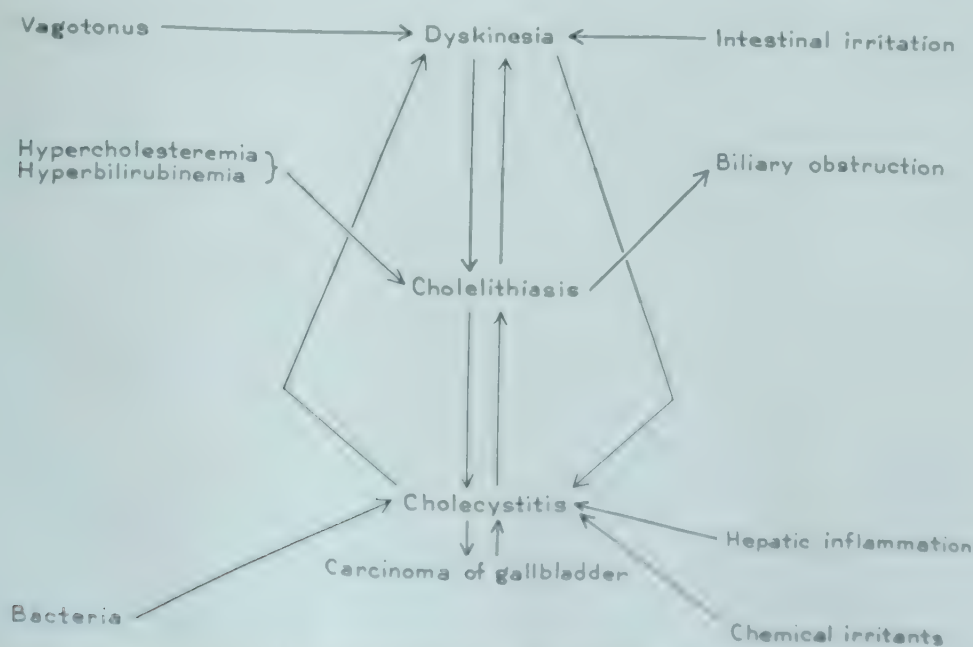


FIG. 128 Interrelation of gallbladder syndromes.

hesions, abscesses, or neuroma formation in the stump also may be present (Fig. 126C and D). Occasionally, because of anomalies of the cystic duct, too long a stump is left, which in time becomes dilated and acts as a gallbladder, even with formation of stones and visualization during cholecystography [1480]. Postoperative pancreatitis explains some instances, while others are caused by stenotic or hypertrophic sphincters not appreciated at surgery, or by carcinomas which were overlooked.

In most instances the symptoms are related to altered physiology, especially when a well-functioning gallbladder is removed. Following cholecystectomy, the sphincter of Oddi remains relaxed for about 6 months [229]. This produces two main sequelae.

1. Constant loss of bile reduces the amounts available for lipid absorption after meals, with resulting clinical symptoms apparently relieved by bile acid administration.

2. The intraduodenal pressure is extended into the biliary duct system, which subsequently dilates. This pressure effect makes itself felt in Bromsulphalein retention and phosphatase elevation in animals [834]. In man effects on hepatic function appear to be only transient [482].

The common duct has been said to dilate gradually and to assume the function of a gallbladder which concentrates bile several months after cholecystectomy, when normal tonus of the sphincter of Oddi is restored. Recent intravenous cholangiographic studies several months after cholecystectomy showed abnormal ducts in about half the patients with symptoms. Dilatation of the ducts and nonvisualization of the terminal portion of the common duct were each noted in about one-fourth of all examinations, while common duct stones and re-formed gallbladders were found in 7 and 3 per cent, respectively [2880]. In one series no difference was found in the caliber of the extrahepatic ducts between asymptomatic control patients and patients with postcholecystectomy symptoms [2107]. Symptoms may reappear which are caused by sphincter of Oddi spasm, particularly in instances where dyskinesia was the primary indication of cholecystectomy. Fat intolerance may be a prominent feature, owing to the cholagogic action of fat. At this time stones may precipitate in the common duct as a result of inspissation in the bile, leading to biliary obstruction and infection and thereby reestablishing the original malady to a considerably more serious degree.



PART III

*Clinical Study of Hepatic
Function and Structure*



The evaluation of hepatic function is of major importance in the diagnosis and management of diseases in an organ system with such diverse functional activity. Practical experience has shown that the functional diagnosis alone is of limited value. This has created the demand for supplementation by the study of structural alterations. This supplementation has become available through the use of liver biopsy. The tests in practical use in the diagnosis and management of hepatobiliary diseases are described in detail, while those procedures which are either obsolete or which have not yet been practically developed, although they appear promising, are omitted or mentioned just briefly. The selection is, by necessity, arbitrary, since it is based chiefly on personal experience.

In practical use the tests are usually applied in a coordinated form, "the liver profile." In such a coordinated system the findings of liver biopsy as well as the alterations in individual diseases are considered. Therefore, the combined use of functional and structural findings in the diagnosis and management of hepatobiliary diseases is discussed in a later chapter (see Chap. 65).

Principles of Hepatic Tests. So-called "liver-function tests" have been in clinical use for over 50 years. More recently, many laboratory findings not directly related to the function of the liver have been used to appraise alterations of its activity. As a result, the actual measurement of the function of the liver or of the biliary tract has often been lost from sight. To clarify a confused picture, definitions of the types of procedures are useful.

ACTIVITY AND TOLERANCE TESTS. A function test theoretically measures activity, capacity, or re-

serve. The tests measuring activity mirror the status of the organ. They usually require a single determination of a substance or a reaction. Tests measuring capacity entail the response to a load of exogenous material such as dyes, whereas tests measuring reserve use the response to a load of endogenous material, such as bilirubin or glucose. Capacity and reserve tests sometimes can not be clearly separated. They represent a response to a specific task, such as the removal of administered galactose, and are best described as tolerance tests and contrasted as a group with the more widely used activity tests. Tolerance tests measuring dynamic response are preferable to activity tests reflecting a static picture.

TRUE LIVER-FUNCTION TESTS. Both activity and tolerance tests of liver function can be subdivided from the point of view of their specificity. A few procedures measure a function which only the liver performs and upon which other organs exert little influence. The substances concerned are either formed or acted upon by the liver. These tests deserve the name "true liver-function tests." Few of the known functions of the liver lend themselves well to clinical practical evaluation, the main exception being the response of the prothrombin time to the administration of vitamin K.

CONDITIONED LIVER-FUNCTION TESTS. The tests which measure functions which the liver shares with other organs or which are influenced by the function of other organs are more numerous. These include tests concerning such processes as serum-protein formation or glycogen deposition in the liver. These tests, therefore, can be called "conditioned liver-function tests." Despite theoretical drawbacks, much practical informa-

tion about hepatic function is obtained from these tests if the influences exerted by other organs are taken into account.

HEPATIC TESTS. To these two groups, a third one, which is the largest at present, must be added. These tests, in contrast to the previous two groups, concern biochemical, serologic, or hematologic data on substances not necessarily formed or acted upon by the liver. They may also concern reactions influenced by functional alterations of the liver. Influences of other organs on these data must also be taken into account. Since a liver function is not necessarily measured by most of the tests used, the term "hepatic tests" is best applied to all tests dealing with the evaluation of the status of the liver [2640].

BILIARY-FUNCTION TESTS. Most of the tests of the extrahepatic biliary system are actually function tests and thus can be called "biliary-function tests."

Classification of Hepatic Tests. Hepatic tests can be classified from the following different viewpoints:

1. A physiologic classification, according to the physiologic basis and referring to the various

metabolic, storage, secretory, excretory, or other functions of the liver.

2. A classification concerning sensitivity and the ability to reflect physiologic fluctuations for clinical or experimental purposes.

3. The practical classification to be applied in the coordinated use of the tests (see Chap. 65).

The potential number of hepatic tests is unlimited, since the liver as the central organ of metabolism influences many body functions. It responds in a characteristic fashion to the administration of many exogenous substances. The physiologist studies the basis of the tests and tries to develop simpler and more specific ones. The clinician or pathologist must select from the large number of hepatic tests the one of greatest practical value in a given situation. A single hepatic test rarely suffices, since all functions of the liver are not equally or simultaneously disturbed in hepatic disorders (dissociation of liver function [2202]) and since abnormal results in one test may be encountered with normal hepatic function or structure. This factor, in addition to the multiplicity of hepatic functions, requires performance and discussion of many tests.

Determinations of the serum-protein fractions are most valuable and widely used as hepatic tests. Determination of the total serum proteins is the basis upon which quantitative fractionation is made.

Total Serum Protein

Physiologic Basis. The liver as the main source of the serum proteins influences the level of total serum protein. In liver diseases, hypoproteinemia would be expected, but no constant relation between hepatic disorders and the total protein levels is found [1074, 2645]. This lack of correlation is explained by the fact that the reduction of the serum-albumin level as a result of hepatic injury is compensated or even overcompensated by elevation of gamma globulin not produced by the hepatic cells. The irregularity of this compensation explains the exceedingly wide range of the total protein level in liver disease. Malnutrition lowers the total serum protein by reducing albumin as well as the globulins [1861].

Methods. Many methods for total protein determination are available. The classic principle of Kjeldahl uses wet digestion in sulfuric acid [1492]. The ammonia thus formed is determined either by distillation or by the principle of nesslerization. In recent years the cumbersome and time-consuming distillation method has been replaced by physical methods, such as estimation of the specific gravity of the serum with the falling-drop apparatus [1672], glass beads, or by flotation in copper sulfate solution, or by determination of the refractive index [3265]. These physical methods are not exact, and chemical methods without digestion, such as the tyrosine method of Greenberg [1835], have been described. The quantitative determination of the

biuret reaction on undigested serum has found the widest acceptance, because it combines rapidity with a degree of accuracy almost matching that of Kjeldahl determinations [1235, 1741, 1763, 1969, 3534]. In various conditions, especially in liver disease, differences are found between the total nitrogen determined by Kjeldahl methods and the biuret reactivity [1764].

Technique. REAGENTS.

1. Distilled water.
2. Biuret reagent according to Weichselbaum [3534]. Prepare an accurately titrated 0.2 N carbonate-free sodium hydroxide solution. Dissolve 45 gm sodium potassium tartrate (Rochelle salt) in 200 ml 0.2 N sodium hydroxide. Add 15 gm copper sulfate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. When the copper sulfate has entirely dissolved, add 50 gm potassium iodide and make up to 1 liter with 0.2 N sodium hydroxide.

PROCEDURE.

1. Unknown: Pipette 0.4 ml serum into a 10-ml graduated mixing cylinder and dilute to 10 ml with distilled water. Mix well by inversion and transfer 5.0 ml to an Evelyn cuvette. Add 5.0 ml biuret reagent and mix well by shaking.
2. Blank: Prepare the blank with 5.0 ml distilled water and 5.0 ml biuret reagent.
3. Readings: Let the solution stand at least 30 minutes. Since the color is quite stable, reading may be deferred for as long as 24 hours. Read on the photoelectric colorimeter or spectrophotometer at a wavelength of 540 m μ .
4. Standard curve to be used for all protein fractions: The standard protein solution may be either of the following:
 - a. A sample of pooled normal human serum, the protein content of which has accurately been determined by the Kjeldahl method.
 - b. Commercial human albumin. Although it is expensive, this has the advantage of a high concentration and permits standards to be prepared with concentra-

tions well in excess of normal serum. The protein content must be determined exactly by the Kjeldahl method.

Using normal saline solution as the diluent, prepare standards with protein concentrations of about 1.0, 2.0, 4.0, 6.0, and 8.0 gm per 100 ml. Determine the protein concentration as described above. Draw the standard curve from the data obtained. This standard curve is used for all determinations using biuret reagent.

Results. The normal values for total serum proteins lie between 6.3 and 7.9 gm per 100 ml, with an average of 7.2 gm [1311, 2572, 2627]. A level below 6.0 gm indicates protein deficiency. In liver diseases the averages are approximately the same; in obstructive jaundice the average is slightly lower than in hepatitis. The range of values in liver diseases is much wider than normal. Values below 4.0 gm have been encountered. Age and sex have little influence on the level, while hemoconcentration increases it.

Evaluation. The practical value of the determination of the total serum protein in diseases of the liver is in appreciating the state of nutrition. It is of particular value in preoperative management. The serum-protein level is not a reliable index of the protein stores, although low levels generally indicate reduced protein depots.

Fractionation of Serum Proteins

The serum proteins are a composite of several fractions, an increasing number of which are being found with improving techniques. The various fractions are apparently formed at different sites, the most important of which is the parenchymal hepatic cell. The fractions respond in different and often opposite ways to liver injury. Some fractions are identified by specific biologic activity, for instance, in blood coagulation, but most of them are differentiated mainly on the basis of molecular weight, size, and shape, rather than on the basis of chemical constitution of specific amino acids. Such properties form the basis of the precipitation methods with heat, salts, metals, or change of pH. Immunologic methods have also been devised to determine single protein fractions quantitatively.

Originally, the plasma proteins were divided into three fractions: (1) The one with the highest molecular weight, fibrinogen, precipitating upon leaving the circulatory bed, (2) albumin, (3) globulin. The protein fraction precipitating after one-half saturation of serum with ammonium

sulfate was called globulin because it was originally thought to be derived from leukocytes (globules). The portion remaining in the solution was called albumin. Variation of the ammonium sulfate concentration made possible a subdivision into pseudoglobulin and euglobulin. This empiric division depends largely upon the molecular size of the protein. Until recently, the chemical methods for serum-protein determinations were based on the division achieved with half-saturated solution of ammonium sulfate, except that properly adjusted concentrations of sodium sulfite or sulfate salts replaced the nitrogen-containing ammonium sulfate [1560].

Electrophoresis. The development of more accurate but also more elaborate physicochemical methods has led to further differentiation of the serum and tissue proteins [1311, 2221]. This was especially prompted by the fractionation of the serum proteins in the preparation of biologic plasma substitutes [616]. Among the many physicochemical methods used, including ultracentrifugation and ultradialysis, electrophoresis is most widely applied. This method records differences in molecular size and shape, expressed as variations in optical densities at the boundaries of layered protein and buffer solutions, which develop when an electric current is sent through them. Optical methods enable photographic recording of electrophoretic curves (Fig. 17). From these curves the relative concentrations of the proteins are determined. The shape of the curves depends on the buffer used [1508]. The method has been simplified by the use of filter paper [1884] or starch [1883] as a medium for the electrophoretic current.

PAPER ELECTROPHORESIS. This zone electrophoresis is now being adapted for use in the clinical laboratory and may replace salt-precipitation methods and empiric flocculation tests. Several techniques have been devised, especially in Europe [1251, 1806]. The proteins spread on the paper strip by the electric current are stained with bromphenol blue or amido black 10B (Fig. 129). The amount of dye is measured either after elution or photometrically with a densitometer, which may even integrate the curves for quantitative determination. The results of zone electrophoresis are not exactly identical to those with free electrophoresis [2134]. Determinations can be made on post-mortem blood specimens [610]. Lipoproteins and glycoproteins can be determined

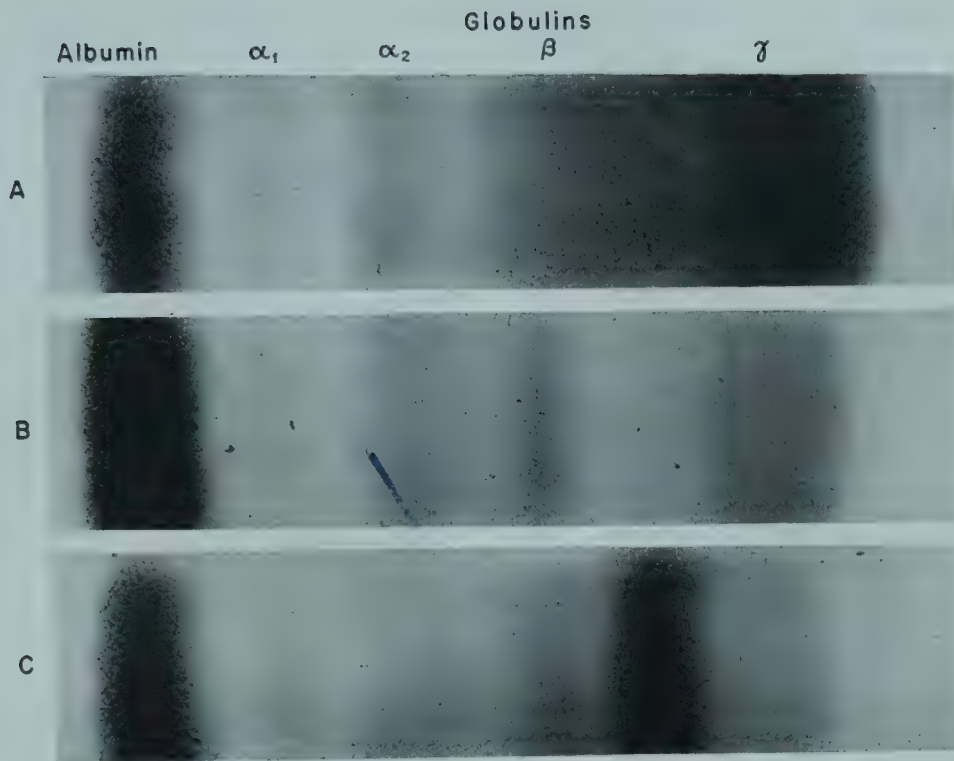


FIG. 129 Paper electrophoresis strips. A. Cirrhosis. B. Normal. C. Myeloma with elevation of a globulin with a motility between alpha and beta globulin.

with the use of fat stains such as oil red O or sudan black, or the PAS reaction, respectively.

ELECTROPHORETIC PROTEIN PATTERNS. On the basis of electrophoresis the serum proteins are separated into a rapidly moving fraction, albumin, and a series of slower ones, alpha, beta, and gamma globulins, the last having the largest molecule of the three. Alpha globulin as determined electrophoretically is not completely precipitated with the globulins in the salting-out method of Howe, based on half saturation with ammonium sulfate. The Howe method results in the albumin fraction appearing greater and the globulin fraction smaller than in the electrophoretic method [615, 2569]. Recent precipitation methods attempt to imitate the electrophoretic partition by proper adjustment of the concentration of the precipitating salts [615, 1742, 1966, 2184, 2230, 2305] (Table 13).

Alpha and gamma globulins have been further subdivided into α_1 and α_2 , and γ_1 and γ_2 fractions. In plasma, a fibrinogen peak is noted between beta and gamma globulin. It is superimposed on the γ_1 peak in plasma, in which this fraction is present, so that occasionally high fibrinogen levels have been recorded in cirrhosis when actually γ_1 globulin was elevated [1073]. The parallel determination of

serum and plasma samples in the same blood reveals the true fibrinogen and γ_1 levels (Fig. 17).

Other methods of separation of protein solutions are based on fractional precipitation with methanol at varying low temperatures [616, 2597, 2683], ultraviolet absorption [1956], or specific

Table 13 Normal Serum-protein Values as Determined by Various Methods, in gm per 100 ml

<i>Serum-protein fraction</i>	<i>Howe method: sodium sulfate</i>	<i>Newer chemical methods based on electrophoretic partition</i>	<i>Electrophoretic partition</i>
Total protein.....	6.0-8.0	6.0-8.0	
Albumin.....	4.6-6.7	3.0-5.0	3.8-5.1
Total globulin.....	1.2-2.3	2.0-3.3	2.2-3.5
A/G ratio.....	2.69	1.2-2.4	1.2-1.8
Alpha globulin.....		0.5-1.5	0.7-1.6
Beta globulin.....		0.5-1.4	0.5-1.2
Gamma globulin...		0.5-1.25	0.5-1.25
Fibrinogen.....		0.3-0.6	0.1-0.5

gravity [2357]. Chemical and physical attempts to partition serum proteins are also reflected in the flocculation and turbidity tests discussed later.

Serum Albumin

Physiologic Basis. Since serum albumin is formed only by the hepatic cells, reduction of serum albumin is an indication of deranged hepatocellular function, if the other factors such as nutrition or urinary protein loss are taken into account. Since the half-life of the serum proteins has been said to be approximately 10 days [3203] and is possibly much longer [101], faulty formation must be present for more than 1 week to reduce the albumin levels significantly.

Methods. The determination of albumin depends on the principle of the albumin-globulin partition. Immunologic methods are the most specific [1179] and can also be used in micro-methods. The electrophoretic methods follow in specificity; zone electrophoresis particularly may become the routine method of the future. Precipitation of albumin by hematin has been recommended as a specific means of determining its level [2821]. Specific gravity methods have not found wide acceptance in spite of their simplicity. For practical purposes, salting-out procedures suffice and some show good correlation with electrophoretic results [1625, 2634]. For the diagnosis of hepatic disorders, the Howe method [1560], which includes part of the alpha globulin as albumin, is not worse than the modern methods, which agree better with electrophoresis. Both the older method of Howe and one of the newer methods, that of Wolfson and Cohn [615], are described.

Technique: Howe Method. REAGENTS.

1. 22 per cent sodium sulfate. Dissolve exactly 22.0 gm anhydrous sodium sulfate in distilled water at 37°C. Make up to 100 ml with distilled water and store in an incubator at 37°.
2. Ether, USP.
3. 0.2 N sodium hydroxide solution.
4. Biuret reagent (see Technique, under Total Serum Protein, this chapter).

PROCEDURE. To 10.0 ml 22 per cent sodium sulfate solution add 0.4 ml serum. Stopper and invert. Add 1.5 cc ether. Stopper again and invert about a dozen times. Centrifuge at high speed, 2,500 rpm, for about 10 minutes.

Slant tube to loosen precipitate from the wall of the test tube. Insert a pipette and remove 5.0 ml clear centrifugate to a clean test tube. Place the tube in water bath at 25°C. Add 5.0 ml biuret reagent. The

blank consists of 5.0 ml sodium sulfate solution and 5.0 ml biuret reagent. Readings are made in 30 minutes at 540 m μ . The standard curve is the same used for total serum protein (see Technique, under Total Serum Protein, this chapter)

CORRECTIONS. To correct for lipemia or icteric discoloration, add 3.0 ml 0.2 N sodium hydroxide solution serum to 3.0 ml of the remaining centrifugate. This is read against a blank containing 3.0 ml sodium hydroxide and 3.0 ml water. The reading is subtracted from the original value. The highest readings for very icteric serums correspond to 0.3 to 0.4 gm per 100 ml.

Technique: Wolfson and Cohn Method. REAGENTS.

1. 28.0 per cent sodium sulfite solution: Dissolve exactly 28.0 gm anhydrous sodium sulfite in distilled water at 28°C. Since the salt is not readily soluble, considerable shaking may be required. Make up to 100 ml with distilled water, and store at room temperature.
2. Span-ether reagent: Mix 1.0 ml Span 20, made by the Atlas Powder Co., Wilmington, Del., with 99 ml ether, USP. Filter through a moderately fast paper into a 100-ml diluting cylinder, and make up to 100 ml with ether. Store in a tightly corked bottle.
3. Biuret reagent (see Technique, under Total Serum Protein, this chapter).

PROCEDURE.

1. Unknown: Place 9.6 ml 28 per cent sodium sulfite solution in a test tube. This solution should not be pipetted, since it has a tendency to crystallize, particularly when in contact with a cold pipette. Small calibrated test tubes marked at 9.6 ml are more convenient. These may be filled rapidly from a large burette. Enough may be prepared at one time for the entire day's determinations.

To the sodium sulfite solution 0.4 ml of serum is added, and the contents of the test tube are mixed by inversion. Add 1.5 ml Span-ether reagent and invert slowly four times. Centrifuge for 5 to 10 minutes at 1,500 to 2,000 rpm. After centrifugation, carefully place a pipette through the Span-ether layer and beneath the packed globulin, slanting the tube to separate the precipitate from the wall. Withdraw 5.0 ml of the clear centrifugate and transfer to a cuvette. Add 5.0 ml biuret reagent and mix well by shaking.

2. Blank: Prepare the blank with 5.0 ml sodium sulfite solution and 5.0 ml biuret reagent.

3. Readings: After letting it stand for at least 30 minutes, read on the photoelectric colorimeter at a wavelength of 540 m μ .

4. Standard curve: See Technique, under Total Serum Protein, this chapter.

Results. With electrophoretic methods, albumin represents from 50 to 65 per cent of the total serum protein [2221]. The concentration ranges from 3.5 to 5.0 gm per 100 ml serum [809, 2221]. The chemical values vary with the Howe method

from 4.7 to 5.7, with an average of 5.2 gm per 100 ml [1311]. Average normal values obtained with the newer methods are 3.8 gm per 100 ml, with 27.2 per cent sodium sulfite [1742], 3.7 to 3.8 gm per 100 ml with 28.0 per cent sodium sulfate [615, 3646], and with methanol [2597] 3.9 gm per 100 ml [1966].

In various diseases not necessarily associated with hepatocellular damage the chemical and electrophoretic values fall below normal, especially if malnutrition or albuminuria is present [2043, 2381, 2627] (Table 14). Much has been written

Table 14 Diagnostic Significance of Serum-albumin Level

- I. Hepatic diseases associated with reduction of serum-albumin level
 - A. Cirrhosis
 - B. Chronic hepatitis
 - C. Prolonged obstructive jaundice
- II. Extrahepatic conditions associated with reduction of serum-albumin level
 - A. Malnutrition and debilitating diseases
 - B. Ascites
 - C. Nephritis, nephrosis
 - D. Chronic gastrointestinal disorders
- III. Hepatic disease in which low serum-albumin level may be the only laboratory clue to the diagnosis
 - A. Cirrhosis without jaundice
- IV. Diffuse hepatic disease in which normal serum-albumin levels frequently occur
 - A. Acute hepatitis
 - B. Early extrahepatic cholestasis

about the changes in hepatic disease [729, 1260, 1671, 2627, 2661, 2695, 2766, 3204, 3205, 3335, 3593]. In acute hepatitis the values are only moderately reduced, but in chronic stages they tend to drop further. The depression is somewhat correlated with the severity of the disease. In chronic hepatic diseases, more so in cirrhosis with jaundice than in cirrhosis without jaundice, the values are very low, the average being below 3.0 gm per 100 ml, and levels below 1.0 gm have been encountered. In asymptomatic cirrhosis, normal values are found. In protracted obstructive jaundice, the values are reduced. In cirrhosis with ascites the serum-albumin level is lower than in the cases without ascites, the difference being statistically significant. In experimental liver damage the serum-albumin level is also reduced [253, 947].

Evaluation. The serum-albumin level is of little help in the differential diagnosis of jaundice, since it may be equally reduced in both obstructive jaundice and hepatitis. Its main value lies in the

follow-up of therapy, especially in cirrhosis, where improvement in the serum-albumin level is the best sign of successful medical treatment [973, 2661, 3205].

Alpha Globulin

Although alpha globulin is probably formed by the hepatic cells, its serum level is not of great diagnostic significance in hepatic disorders because of the wide range of values and of the technical difficulties of determination. Chemical partition so far does not permit a reliable determination of alpha globulin [2627, 2634], although paper electrophoresis may make it a practical clinical test. The normal values lie between 0.8 and 1.1 gm per 100 ml and may be reduced to 0.1 gm and elevated to 1.7 gm in hepatic diseases [2627]. The extremely low levels are found in fatal hepatic failure. Active infection or body injury increases the level. Some lipoproteins migrate with alpha globulin; in most liver diseases this migration is reduced, except in chronic viral hepatitis, where it is increased [1068].

Beta Globulins

The normal values lie between 0.9 and 1.2 gm per 100 ml serum, with elevations to 1.7 gm in xanthomatous biliary cirrhosis and reductions to 0.5 gm in cirrhosis. Most of the lipoproteins migrate electrophoretically with the beta globulins. In general they are low in infection and high in lipemia.

The beta globulins show some correlation with the degree of cholestasis but not with the degree of hepatocellular degeneration, the bilirubin level, or any serum-lipid fractions.

Paper electrophoresis, especially if combined with the demonstration of lipoproteins, possibly may make beta globulin determinations meaningful in the diagnosis of hepatobiliary diseases.

Gamma Globulin

Physiologic Basis. Gamma globulin is not formed by the hepatic cells but by mesenchymal elements such as plasma cells and reticuloendothelial cells. The Kupffer cells and mesenchymal cells in the portal tracts possibly form it, as well as infiltrating histiocytes and plasma cells. The elevation of gamma globulin in hepatic disorders can be related to three factors: (1) stimulation of hepatic mesenchymal cells; (2) infiltration by plasma cells and reticuloendothelial cells; (3) excess formation outside the liver caused by stimula-

tion of extrahepatic mesenchyma, or by utilization for gamma globulin formation of amino acids, which are in excess because they are not used by the damaged liver for albumin synthesis. The last-named factor may explain the reciprocal behavior of serum albumin and gamma globulin [895]. Gamma globulin is elevated not only in hepatic conditions but also in many other diseases, especially those associated with proliferation of the reticuloendothelial system, hyperimmune reactions, and chronic infection [895, 1311, 2221]. In severe obstructive jaundice, gamma globulin elevation develops slowly, either because bile-laden Kupffer cells cannot produce it or because of a less conspicuous mesenchymal reaction. The electrophoretic separation of gamma globulin into γ_1 and γ_2 fractions often occurs in hepatic diseases but is of little diagnostic significance [3593].

Methods. Chemical methods have been described, based mainly on sulfate-salt precipitation, which imitate the standard electrophoretic separation [1624, 1742, 3646]. Simple turbidimetric methods [1082, 1569, 1966, 2869] are also well correlated with electrophoretic determinations. These methods permit serial gamma globulin determinations routinely.

Technique for Turbidimetric Method. This method is based on the salt fractionation of Wolfson and Cohn [615], but a precipitate rather than a filtrate is analyzed turbidimetrically [1569]. The serum should be centrifuged at high speed before use so that extraneous material, such as a few erythrocytes or small fibrin shreds, does not interfere with the determination.

REAGENTS.

1. Ammonium sulfate-sodium chloride solution. In a 1-liter volumetric flask, dissolve 189 gm ammonium sulfate in about 500 ml distilled water. Add 29.3 gm sodium chloride, dissolve, and make up to 1 liter with distilled water. Store at room temperature.

PROCEDURE. Pipette 5.0 ml ammonium sulfate-sodium chloride solution into a test tube and add 0.1 ml serum. Mix by careful, slow, repeated inversion. Continue mixing until, within a minute or two, the gradually developing visible turbidity has reached a maximum. After 30 minutes, read at a wavelength of 650.

Standard curves can be made either by using dilutions of commercial gamma globulin or by comparing the readings with chemical or electrophoretic determinations of gamma globulin. The standard curve for other turbidimetric readings can also be used, and the values obtained can be expressed either in units or in grams per 100 ml after conversion with one of the published formulas [1569].

Results. The normal gamma globulin concentration is between 0.6 and 1.25 gm per 100 ml serum for the turbidimetric [1569], colorimetric [1624, 3646], and the electrophoretic methods [2221, 2627]. Electrophoretically, gamma globulin normally averages approximately 11 per cent of the total serum protein. The good correlation between the results of the electrophoretic and turbidimetric or colorimetric methods holds true mainly for normal serum or for that from patients with hepatic or chronic infectious diseases or hyperimmune conditions, but not for serum from patients with multiple myeloma or chronic nephritis. Since salt partitions precipitate proteins other than gamma globulin and leave some gamma globulin in solution [1570], the two errors may cancel themselves out.

Gamma globulin frequently is elevated in hepatic disease of almost every type [1260, 2228, 2627, 2636, 2695, 2766, 3149, 3204, 3205]. In viral and toxic hepatitis, the elevation is moderate and depends somewhat upon the severity of the disease (Table 15). In chronic viral hepatitis the level does not necessarily drop when recovery from the acute symptoms occurs and results of other hepatic tests return to normal. The continued elevation is a sign of mesenchymal activity and may herald a transition into cirrhosis. In cirrhosis, gamma globulin levels are frequently high, especially when jaundice is present. They sometimes

Table 15 Serum-Gamma Globulin Level in Various Hepatobiliary Diseases

Diagnosis	No. cases	Mean, gm/100 ml	Range, gm/100 ml	% abnormal
Normal.....	283	0.91	0.61-1.43	3.2
Control patients...	106	1.06	0.70-1.47	16.7
Acute hepatitis....	433	1.64	0.75-3.90	84.6
Cirrhosis with jaundice.....	312	2.05	0.73-6.86	92.0
Cirrhosis without jaundice.....	219	1.91	0.80-5.40	89.5
Extrahepatic biliary obstruction.	165	1.41	0.68-2.70	62.0
Xanthomatous biliary cirrhosis....	8	2.41	1.29-3.68	100.0

Sources: Method—de la Huerga and Popper [1569]; data—de la Huerga and Popper [1569], MacLagen *et al.* [2149], Popper *et al.* [2636], Sophian and Connolly [3136], Spellberg *et al.* [3149], unpublished data [3394].

return to normal on treatment but are often elevated in inactive forms of cirrhosis. In fatty liver the serum-gamma globulin level is usually normal, but in the presence of jaundice it sometimes rises. The highest values are encountered in postnecrotic cirrhosis. Elevation above 4.0 gm per 100 ml serum is almost diagnostic of postnecrotic cirrhosis in a patient with liver disease. In extrahepatic cholestasis, a slight elevation is frequently found which detracts from the diagnostic value of gamma globulin determinations in the differentiation of medical and surgical jaundice. The elevation is greater in prolonged and infected cholestasis, possibly because of increased hepatic gamma globulin formation. In primary hepatic carcinoma the serum-gamma globulin level is high, while in secondary carcinoma of the liver it is frequently normal, and in lymphomas involving the liver it may be low.

The elevated gamma globulin level in nonspecific reactive hepatitis may be the result of the underlying disease rather than of the hepatic changes. In apparently healthy blood donors, moderate elevation is occasionally found [1569], possibly a reflection of the carrier state of hepatitis. In malnutrition, gamma globulin is low [1861]; it often rises above normal in the recovery period [1216].

Table 16 Diagnostic Significance of Serum-Gamma Globulin Level

- I. Hepatic diseases associated with elevated gamma globulin level
 - A. Cirrhosis, especially postnecrotic (most severe)
 - B. Prolonged hepatitis
 - C. Infected extrahepatic cholestasis
 - D. Hepatocellular carcinoma
- II. Extrahepatic conditions associated with elevated gamma globulin level
 - A. Chronic inflammation
 - B. Collagen disease
 - C. Tuberculosis
 - D. Myeloma (severe in some)
 - E. Sarcoidosis
 - F. Kala-azar and other tropical diseases
 - G. Carcinoma and Hodgkin's disease (irregularly)
- III. Hepatic diseases in which elevated gamma globulin level may be the only laboratory clue to the diagnosis
 - A. Cirrhosis without jaundice
 - B. Transition of hepatitis into cirrhosis
- IV. Diffuse hepatic diseases in which normal gamma globulin levels frequently occur
 - A. Acute hepatitis
 - B. Early cholestasis (intrahepatic and extrahepatic)
 - C. Hepatic amyloidosis

Many extrahepatic disorders are associated with increased serum gamma globulin (Table 16). Elevation of the serum-gamma globulin level out of keeping with the clinical manifestations or other laboratory findings should raise the suspicion of a nonhepatic disease, such as myeloma.

Evaluation. Elevation of gamma globulin is probably the most frequently encountered abnormal result obtained in any of the hepatic tests [3149]. Because of the incidence of slight elevations in apparently healthy people and greater elevations in many diseases, the diagnostic value of elevated serum-gamma globulin level is small. The slight elevation in extrahepatic cholestasis reduces the value of the test in the differential diagnosis of jaundice. It is of value in the differentiation between acute hepatitis, in which it is slightly elevated, and cirrhosis, in which it is usually greatly elevated. It assists in the recognition of the transition of acute hepatitis into cirrhosis. It is characteristically very much elevated in postnecrotic cirrhosis. Absence of gamma globulin elevation in the presence of jaundice speaks for extrahepatic biliary obstruction, fatty liver with hepatic failure, metastatic carcinoma, or lymphoma.

Total Serum Globulin

Since the total serum-globulin level is the sum of the various globulin components, its diagnostic value depends upon the method of determination. In general, in liver disorders, gamma globulin rises and alpha globulin drops, whereas beta globulin behaves erratically. The rise of the total globulin in liver diseases is usually more apparent with Howe's salt precipitation than with electrophoresis or with those salt fractionations which simulate electrophoretic partition.

Globulin values are obtained by subtracting the albumin level from the total serum protein. The absolute values by electrophoresis as well as those obtained by salt-precipitation methods imitating it, are normally between 2.5 and 3.5 gm per 100 ml, with a mean of 2.7. With Howe's method the normal values are between 1.3 and 2.4 gm per 100 ml, with a mean of 2.0 [1311]. Abnormal reduction is encountered in amyloidosis and nephrosis. An increase is found in almost all liver diseases and in many other conditions, such as multiple myeloma, chronic infections, leukemia, and dehydration, which raise the gamma and beta globulin levels [1378]. This increase can be caused either by an elevation of beta globulin, more pronounced in obstructive jaundice, or by an elevation of

gamma globulin, more pronounced in hepatitis or cirrhosis. Consequently, the elevation of total globulin level has little differential diagnostic significance in the various hepatic disorders.

Euglobulin; Pseudoglobulin. With salt-precipitation methods, globulins have been separated by variations of the salt concentration. Euglobulin is the fraction insoluble at relatively low salt concentrations, in contrast to pseudoglobulin [1560, 2221]. A similar fraction is the 13½ per cent globulin [729] precipitated by that concentration of sodium sulfite. This fraction occurs mainly under abnormal circumstances and resembles the electrophoretic gamma₁ globulin. The modern electrophoretic and chemical methods have obviated the use of these fractions.

Albumin/Globulin Ratio

The albumin/globulin ratio had been popular long before the modern subdivision of the various protein fractions. Its main advantage is its independence of hemoconcentration or hemodilution. Its changes in liver disease are mathematically more impressive than the determination of the individual proteins because of the simultaneous drop in albumin and increase in globulin. This is outweighed by its disadvantages. A reduction of the ratio can be caused by either an increase in globulin or a reduction in albumin, each having a different significance. Moreover, the various globulins have different and often divergent tendencies, obscuring the interpretation.

RESULTS. With the method of partition according to Howe, which is the most widely used [1560], the mean A/G ratio is 2.70 (Table 13). A value below 1.25 is abnormal.

Theoretically, the partition by electrophoretic methods is more sound. Since part of the alpha globulin is determined with albumin in Howe's method, the electrophoretic A/G ratio is considerably lower, as are the ratios obtained by partition methods imitating electrophoresis [2229, 2627]. The mean is 1.7 and only a level below 1.0 should be considered abnormal [2627].

The albumin/globulin ratio is reduced in all types of liver diseases, especially in cirrhosis with jaundice (Table 17). In extrahepatic cholestasis the reduction is approximately the same as in acute hepatitis. Since the alpha globulin level acts similarly to albumin and antagonistically to the other globulin fractions, alterations characteristic of hepatic disorders are reflected better in the Howe partition than in the electrophoretic parti-

tion. If any diagnostic advantage is to be derived from the A/G ratio, the Howe partition is slightly superior in differentiating hepatobiliary diseases

Table 17 Albumin/Globulin Ratios in Various Hepatobiliary Diseases

Diagnosis	No. cases	Mean	Range	% abnormal
Normal.....	83	2.70	1.48-3.64	0
Acute hepatitis....	272	1.51	0.56-2.85	30.5
Cirrhosis with jaundice.....	402	0.84	0.22-2.55	87.5
Cirrhosis without jaundice.....	232	1.29	0.30-2.80	55.2
Biliary obstruction..	96	1.57	0.51-3.10	23.6
Xanthomatous biliary cirrhosis.....	8	2.90	0.52-4.50	7.2

Sources: Method—Howe [1560]; data—Armas-Cruz *et al.* [96], Howe [1560], Kibrick and Clements [1743], Popper *et al.* [2627], Ricketts *et al.* [2761], Ricketts and Wissler [2767], Shay and Harris [3028], Spellberg *et al.* [3149], Thompson and McCabe [3326], unpublished data [3394], Watson and Hoffbauer [3510], Welin [3554].

from other disorders, acute hepatitis from cirrhosis, and especially extrahepatic obstruction from acute hepatitis. However, some seem to prefer electrophoretic partition [2695]. The albumin/gamma globulin ratio [2228], determined by simple chemical partition, was calculated [2634]. It is theoretically more sound and would deserve further clinical evaluation.

EVALUATION. If the A/G ratio is used for diagnostic purposes in liver disease, the Howe partition is slightly better, despite its theoretical disadvantage. The divergent tendencies of the various globulin fractions explain why significant variations in the individual globulins are often not reflected in the A/G ratio [1260].

Fibrinogen

The plasma normally contains between 0.2 and 0.4 gm fibrinogen per 100 ml. It is reduced occasionally in severe hepatic failure and sometimes elevated in cirrhosis. It is often elevated in chronic infections such as tuberculosis. It also varies greatly under normal circumstances. Furthermore, the presence of fibrinolytic enzymes, supposedly also formed by the liver, further detracts from any diagnostic usefulness in hepatic disorders.

NONSPECIFIC SERUM-PROTEIN REACTIONS: FLOCCULATION OR TURBIDITY TESTS

A series of simple nonspecific serum tests was described on an empirical basis originally, without knowledge of the underlying physicochemical alterations. These tests have in common an alteration of the solubility of various serum colloids under properly adjusted conditions. The type of alteration, as well as the conditions, varies from test to test. Subsequently the physicochemical bases of many of them were elucidated and similar tests with known basic principles were described. Three types of tests are differentiated: (1) modifications of salting-out procedures which change internal ionization of proteins, such as gamma globulin or distilled water-turbidity tests; (2) formation of insoluble protein complexes with electronegative colloids, with adherence of protein to particle surfaces, as in the colloidal gold test; to metallic salts, as in the zinc sulfate-turbidity test; or to organic compounds, as in the thymol-turbidity test; (3) testing the stability of emulsions which readily precipitate and in doing so combine with gamma globulin, as in the cephalin-cholesterol-flocculation test.

The tests, despite their nonspecific nature, lend themselves well to diagnostic applications in a system in which the results supplement one another. These serum reactions have become the most widely used of the hepatic tests, although they do not really mirror hepatic function.

Factors Influencing the Results of Nonspecific Protein Reactions

Difficulties in interpreting results of the flocculation tests are increased by an inadequate knowledge of the serum proteins and by the possibility that electrophoretically uniform proteins are in reality heterogeneous. Nevertheless, several probably oversimplified basic factors can be recog-

nized, the influence of each varying in different tests. In almost all the serum-protein reactions under consideration, stabilizing and precipitating factors exist. The stabilizing effect is illustrated by the observation that normal serum precipitates a cephalin-cholesterol suspension if it is sufficiently diluted, since the stabilizing effect disappears earlier during dilution than the precipitating effect [1374]. In almost all the tests, an increase in gamma globulin facilitates precipitation [3666] and, in addition, possible qualitative alteration of gamma globulin may have a similar effect [1374]. Albumin seems to exert a stabilizing effect, inhibiting flocculation [182, 1671, 2147], and again qualitative rather than quantitative differences have been claimed to explain decreased inhibition by abnormal serum [2148]. Alpha₁ globulin, rather than albumin, has been stated to be the stabilizing factor. This factor is very labile and quickly disappears in acute hepatic failure, thus explaining abnormal reactions before serum albumin is reduced [1374]. In some of the tests, at least, such as the thymol-turbidity test, beta globulin seems to exert a precipitating effect [182, 609].

In many tests, flocculation or turbidity is inhibited by a depression factor which is increased in any type of cholestasis. The same stabilizing effect is obtained by the addition of human gallbladder bile to serum, increasing the level of bilirubin to that encountered in severe biliary obstruction [853, 2636]. The addition of corresponding amounts of lecithin has a similar inhibitory effect. This depression factor is not equally effective in all tests. It is particularly outspoken in the zinc sulfate-turbidity test, and it is also found in the thymol-turbidity test and the cephalin-flocculation test [2636]. It is not noticeable in the gamma globulin-turbidity test.

Different factors are involved in the same tests in different stages of the disease. For instance, in hepatitis thymol turbidity is abnormal early owing to a decrease in stabilizing factors, and in later stages it is abnormal owing to elevation of gamma globulin.

Standard Curves

Some tests are based on an arbitrary grading of flocculation, while others depend on the measurement of a turbidity by comparison with arbitrary standards. Most turbidity results are read on the same standard curve. A number of standards exist for making a calibrated curve. Their variations in different laboratories explain discrepancies in results.

Turbidity Standards. The original standards were devised for the visual comparator. They are not suited for photoelectric instruments and did not make corrections for lipemic or extremely icteric serum [853]. Precipitation of known amounts of protein with sulfosalicylic acid has been suggested [853, 2147], but results are not reproducible [853]. The method now most widely used for photoelectric measurements is based on precipitation of barium chloride by sulfuric acid [3019]. The results read on standard curves based on the optical density of turbid suspensions can be compared with each other even if different types of instruments are used. In the published version of the barium sulfate method, a 0.962 *N* barium chloride solution was described as a result of a typographical error. Since a 0.0962 *M* solution was the one intended, results based on the published standard curve are twice as high as the units originally described for any of the tests [853]. Great confusion exists in the literature as to reported values that should be qualified by a statement of the standard used. The use of barium sulfate has been challenged, because the suspension is not necessarily reproducible [853]. This led to the development of standard curves based on the optical density of colored solutions, notably Evans blue dye [1879] and copper sulfate [853]. These were particularly adapted for use on the Evelyn colorimeter, but results obtained with the colorimeter differ from results obtained on the spectrophotometer, as do results obtained on different types of spectrophotometers. Recently a permanent suspension of ground glass indefinitely stable in a sealed cuvette has been recommended as a standard. With whatever standard used, at least three different parallel curves should be

made, and care should be taken to keep the temperature constant [1105].

Technique. The original method described [3019] uses a turbid solution as a stock suspension, which is a possible source of error. The procedure has been simplified. All reagents and flasks are kept at 10°C before mixing.

REAGENTS.

1. Dissolve 1.057 gm barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) in distilled water in a 100-ml volumetric flask and make the volume up to the mark.

2. 0.2 *N* sulfuric acid (H_2SO_4).

PROCEDURE. To each of four 100-ml and four 250-ml volumetric flasks, add exactly 3.0 ml of the barium chloride solution. Then cool these flasks to 10°C and add 0.2 *N* H_2SO_4 , also at 10°C, to each flask to make the volume up to the mark. Invert the flasks several times and make readings from each one at a wavelength of 650 with a small aperture. The average of the readings from the 100-ml flasks represents 20 units, and the average of those from the 250-ml flasks, 8 units. Plot the two points on semilogarithmic paper; the line through them represents the standard curve.

Zinc Sulfate-turbidity Test

Physiologic Basis. Serum proteins, especially the large globulin fraction, are precipitated by dilution of the serum with metallic salt solutions of low ionic strength. After experimenting with various salts, a simple turbidimetric test using barbitol-buffered zinc sulfate was developed to estimate elevations of serum gamma globulin [1875, 1877]. The turbidity is recorded in arbitrary units. The protein precipitated is mainly gamma globulin [1875], although the nitrogen content of the precipitate does not necessarily parallel the turbidity produced [1570]. The turbidity depends on factors, other than the gamma globulin concentration, which disturb the correlation between the results of this test and those of electrophoresis. The serum albumin exerts a stabilizing effect. The turbidity of the same amount of gamma globulin is greater with lower albumin concentrations [1570, 1875]. Ingestion of lipids increases the turbidity [2644]. Nevertheless, the results of this test show a fair correlation with electrophoretic [1570] or chemical [2930] determinations of gamma globulin, particularly in the elevated range encountered in most hepatic disorders. In lower ranges, under normal circumstances or in cholestasis the precipitate is much less than would be expected from the protein concentration. A conversion factor [1877] corrects for this disparity, but it produces a serious discrepancy in higher

ranges. Expression of results in units exaggerates low values when the gamma globulin concentration is only slightly reduced [1570, 2636].

The influence of the depression factor in cholestasis (see Factors Influencing the Results of Non-specific Protein Reactions, earlier in this chapter) is most significant in this test.

Technique. REAGENTS.

1. Zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), 24 mg
2. Barbitol, 280 mg
3. Sodium barbitol, 210 mg

BUFFERED TEST SOLUTION. Dissolve the zinc sulfate, barbitol, and sodium barbitol in a liter volumetric flask with distilled water and make up the volume to 1.0 liter. Keep the solution at room temperature. The pH is 7.6 and should be checked weekly.

PROCEDURE. Dilute 0.1 ml serum to 6.0 ml with the buffered test solution. Allow this to stand 30 minutes and then read in a spectrophotometer at a wavelength of 650 or in a colorimeter with a red filter. Results are expressed in units obtained from the standard curves described.

Results. With the standard curve based on the 0.0962 N barium chloride solution, the upper limit of normal has been considered 15 units, representing 1.3 gm gamma globulin per 100 ml, according to Kunkel's formula [1877]. Sixteen units has been taken as the upper limit of normal by others [2181, 2930]. No lower limit of normal is described. The serum of a small percentage of apparently healthy blood donors gives higher values, some even as high as 25 units. Increased zinc sulfate turbidity does not occur exclusively in hepatic disorders but is found in all diseases in which serum gamma globulin is increased [2180] (Table 18) (see Table 16). In hepatic diseases

Table 18 Diagnostic Significance of Zinc Sulfate Turbidity

I. Hepatic diseases associated with increased zinc sulfate turbidity	Same as for serum gamma globulin (see Table 16)
II. Extrahepatic conditions associated with increased zinc sulfate turbidity	
III. Hepatic diseases in which increased zinc sulfate turbidity may be the only laboratory clue to diagnosis	
IV. Diffuse hepatic diseases in which normal zinc sulfate turbidity frequently occurs	
V. Conditions with jaundice in which zinc sulfate turbidity is normal (or even decreased)	
A. Extrahepatic cholestasis	
B. Intrahepatic cholestasis	
C. Hemolytic jaundice	
D. Familial nonhemolytic jaundice	

the greatest elevation occurs in cirrhosis, especially with jaundice, with levels usually above 25 units [3184] (Table 19). The elevation is less in acute

Table 19 Results of Zinc Sulfate-turbidity Test in Hepatobiliary Diseases

Diagnosis	No. cases	Mean, units	Range, units	% abnormal
Normal.....	101	7.8	2.0-14.0	3.1
Control patients...	165	9.4	1.0-23.0	6.3
Acute hepatitis....	502	19.5	6.0-45.0	65.0
Cirrhosis with jaundice.....	268	27.1	5.1-95.6	92.4
Cirrhosis without jaundice.....	192	20.3	5.2-57.6	71.3
Extrahepatic biliary obstruction.	203	10.8	1.9-35.4	28.1
Hepatic tumor metastases.....	8	12.5
Xanthomatous biliary cirrhosis....	25	20.5	14.0-44.0	100.0
Miscellaneous non-hepatic diseases..	193	44.5

Sources: Method—Kunkel [1875]; data—Kunkel *et al.* [1877], MacLagen *et al.* [2149], Sborov and Keller [2897], Schmid [2930], unpublished data [3394].

hepatitis but may persist longer than any other abnormal laboratory finding, possibly indicating transition into chronic stages [1877]. In contrast, in extrahepatic biliary obstruction the zinc sulfate turbidity is usually normal or low [2181, 3184]. Very low values, down to two units, are seen in extrahepatic or intrahepatic cholestasis, probably as an expression of the depression factor, since the gamma globulin level is not correspondingly reduced. A discrepancy between a normal gamma globulin turbidity and a distinctly reduced zinc sulfate turbidity in the presence of jaundice points to intrahepatic or extrahepatic cholestasis, rather than to hepatitis or cirrhosis [2636]. Only in the presence of severe bacterial infection or prolonged duration of the obstruction, with transition into a cholangitic cirrhosis, or in cholangiolitic cirrhosis is zinc sulfate turbidity elevated in extrahepatic biliary obstruction or intrahepatic cholestasis.

Evaluation. The results of the zinc sulfate-turbidity test mirror either mesenchymal reaction by elevation or the depression factor by reduction. A normal or even reduced zinc sulfate turbidity in the presence of jaundice points to extrahepatic biliary obstruction or intrahepatic cholestasis, or

"cholangiolitis." Zinc sulfate turbidity is elevated in many hepatic and nonhepatic diseases and is of less diagnostic value except in the separation of hepatitis from cirrhosis and in recognition of the transition between hepatitis and cirrhosis.

Cephalin-Cholesterol-flocculation Test

Physiologic Basis. The cephalin-cholesterol-flocculation test determines the stability of a cephalin-cholesterol emulsion following dilution in serum [1375]. Increased amounts of gamma globulin from any cause reduce the stability of the mixture, whereas albumin [1671, 3666] and alpha₁ globulin [1374, 2148, 3666] exert a stabilizing effect against flocculation. The cephalin flocculation does not simply mirror the albumin/gamma globulin ratio [2147, 2149]. Flocculation therefore results from qualitative changes in albumin that reduce its flocculation-inhibiting property or, more likely, from reduction of alpha₁ globulin, which also inhibits flocculation.

Since this globulin fraction formed by the hepatic cells disappears more rapidly than albumin, results of the test become abnormal early in liver injury, especially in hepatitis. Results are abnormal in hepatocellular degeneration of longer than 3 days' duration, while albumin and gamma globulin are still normal. Therefore, the cephalin-flocculation test more specifically indicates liver damage than do other serum reactions that depend more on gamma globulin levels, such as the zinc sulfate-turbidity test. The depression factor caused by cholestasis probably also influences cephalin flocculation. The flocculation is not influenced by extraction of the serum by lipid solvents or by alteration of the physical state of the lipids by heparin [2726].

Method. This simple test is based upon the arbitrary grading of the flocculation, with readings after 48 or preferably 24 hours [1375, 2410]. Several well-standardized mixtures are commercially available. The fresher the cephalin-cholesterol mixture, the more sensitive the reaction [1378]. The reaction is light sensitive [2416]. The lack of agreement in results of different laboratories resulted in many modifications, such as the replacement of cholesterol by desoxycholic acid [1785]. A micromethod has been described [1860]. Recently tubes of desiccated antigen have been made available for performing single tests. Use of several serum dilutions has been recommended [423, 1107]. The cholesterol in the precipitate [2868], the turbidity in the supernatant

fluid [1744], and the amount of reagent precipitated [1639] have been quantitated. The results of these modifications are not significantly different from those of the original simple methods, and in most institutions the original test is performed.

Technique. REAGENTS.

1. Cephalin-cholesterol mixture commercially prepared in vials containing 100 mg partially oxidized cephalin and 300 mg cholesterol

2. Ether USP

PREPARATION OF EMULSION. Prepare a stock solution by placing 8.0 ml ether in the vial containing the cephalin-cholesterol mixture. The solution is stable if kept in a refrigerator. Prepare an emulsion by adding 1.0 ml stock solution to 35 ml freshly distilled water at 65°C. Stir the emulsion well and bring the mixture to a slow boil. Allow it to simmer until the volume is reduced to about 30 ml. Cool the emulsion and use only on the day prepared.

PROCEDURE. Place 1.0 ml emulsion in a centrifuge tube containing 0.2 ml serum diluted in 4.0 ml *N* saline solution. Shake thoroughly and stopper lightly with cotton. Allow the tube to stand undisturbed in total darkness for 24 hours. Since the reaction is photosensitive, neither the serum nor the emulsion should be exposed to strong light, natural or artificial.

Readings are made after 24 hours, and the results are graded from 0 to 4+. A 4+ reaction indicates a clear supernatant fluid with complete flocculation. A ± reaction indicates minimal flocculation. The intermediary stages are graded accordingly.

A control tube with 4.0 ml serum and 1.0 ml emulsion, without serum, should be made to test the stability of the emulsion.

Results. With the methods usually employed, flocculation is rarely noted within 24 hours in normal persons [2814, 3674]. A flocculation of 1+ must therefore be considered as abnormal, especially if used for screening purposes, although for other purposes 1+ is not abnormal.

HEPATITIS. In acute viral hepatitis, abnormal cephalin flocculation is found in a very high percentage of instances, varying in different series between 80 and 90 per cent [3184] (Table 20). In milder forms the incidence is lower. In mild anicteric cases, as well as in intrahepatic cholestasis, or "cholangiolitis," normal results may be found. In chronic forms abnormal cephalin flocculation is very common. In hepatitis induced in volunteers, it is negative in the preicteric period, becomes positive in the icteric phase, and returns to normal relatively early [1427]. In toxic hepatitis, especially from chemicals such as arsenic [1377, 1777], normal results are common.

CIRRHOSIS. In cirrhosis with jaundice abnormal results are found in a high percentage of cases [1378, 3513], but occasionally normal results are

Table 20 Results of Cephalin-Cholesterol-flocculation Test in Hepatobiliary Diseases

<i>Diagnosis</i>	<i>No. cases</i>	<i>Mean</i>	<i>Range</i>	<i>% abnormal</i>
Normal.....	916	0.3+	0-1+	1.6
Control patients.....	2,057	0.3+	0-2+	8.8
Acute hepatitis.....	998	2.8+	0-4+	84.4
Cirrhosis with jaundice.....	336	3.1+	0-4+	88.2
Cirrhosis without jaundice.....	311	1.6+	0-4+	51.0
Extrahepatic biliary obstruction.....	633	1.1+	0-4+	31.7
Xanthomatous biliary obstruction.....	34	2.6+	0-4+	71.5
Hepatic tumor metastases.....	227	1.1+	0-4+	52.3
Chronic passive congestion.....	394	0-4+	54.0
Miscellaneous non-hepatic diseases....	193	0-4+	32.4

Sources: Method—Hanger [1375]; data—Felder *et al.* [990], Mendelsohn and Bodansky [2267], Popper and Schaffner [2640], Ricketts and Wissler [2767], Shay and Harris [3028], Thompson and McCabe [3326], unpublished data [3394], Watson and Hoffbauer [3510].

seen in even severe cirrhosis. In cirrhosis without jaundice, the results are erratic, being abnormal in about half the cases, probably depending upon the degree of hepatocellular degeneration. Under energetic therapy the results of the flocculation tests may become normal [2760]. In uncomplicated fatty metamorphosis of the liver, cephalin flocculation is usually normal [3700], while in fatty liver with jaundice, it is abnormal.

CHOLESTASIS. In intrahepatic and extrahepatic cholestasis, in early stages or without superimposed bacterial infection, cephalin flocculation is rarely 2+ or above [2814, 3513]. Prolonged biliary obstruction often leads to abnormal results [2027], yet no flocculation is noted in some patients dying from hepatic insufficiency in prolonged complete biliary obstruction, despite severe histologic alterations of the liver. Acute bacterial infections complicating extrahepatic cholestasis cause flocculation even in early stages [2632]. Thus in calculous diseases of the biliary tract a

previously negative cephalin flocculation becomes abnormal if clinical signs of septicemia appear.

OTHER DISEASES. The cephalin flocculation is also commonly abnormal with hepatic tumor metastases [2267, 2640] or in chronic passive congestion from any cause [990, 1782]. In infectious mononucleosis abnormal results are typical [417, 614, 1661]. About 20 per cent of patients with diseases of the gastrointestinal tract without obvious hepatic involvement have abnormal results, owing possibly to clinically inconspicuous liver involvement. Abnormal results in other diseases not referable to the liver occur erratically (Table 21). The

Table 21 Diagnostic Significance of Cephalin-Cholesterol Flocculation

- I. Hepatic diseases associated with increased cephalin-cholesterol flocculation
 - A. Hepatitis
 - B. Cirrhosis
 - C. Infected and prolonged extrahepatic cholestasis
 - D. Carcinoma metastases
 - E. Fatty liver with jaundice
- II. Extrahepatic conditions associated with increased cephalin-cholesterol flocculation
 - A. Infectious mononucleosis
 - B. Malaria
 - C. Inflammatory diseases with elevated level of gamma globulin (infrequent)
- III. Hepatic disease in which increased cephalin-cholesterol flocculation may be the only laboratory clue to the diagnosis
 - A. Anicteric hepatitis
- IV. Diffuse hepatic diseases in which normal cephalin-cholesterol flocculation frequently occurs
 - A. Cholestasis (intrahepatic and extrahepatic)
 - B. Inactive cirrhosis without jaundice
 - C. Toxic hepatitis
 - D. Fatty liver

flocculation found occasionally in tuberculosis and rheumatoid arthritis can be explained by increased gamma globulin. In both acquired [2313] or induced [1317] malaria, cephalin flocculation is frequently abnormal.

Evaluation. Statistically, cephalin flocculation shows the best correlation with hepatic-cell degeneration of all the hepatic tests studied [1074, 2645]. Although it does not measure any defined hepatic function, it is one of the most useful tests for the recognition of hepatic-cell degeneration in hepatitis or cirrhosis and for the differential diagnosis of jaundice. No significant flocculation occurs in extrahepatic biliary obstruction and in intrahepatic cholestasis except in the presence of a superimposed bacterial infection or after very protracted

jaundice has led to cirrhosis formation. In screening for liver damage it is useful because of the relatively low incidence of false positive reactions in normal individuals. The test is of little use experimentally, because serums from most laboratory animals normally cause flocculation [510].

Thymol-turbidity Test

Physiologic Basis. In the thymol-turbidity test the turbidity which develops after the dilution of serum with a barbital-buffered thymol solution is recorded [2147]. The precipitate formed consists of a protein-thymol-phospholipid complex [2147], the protein being mainly gamma globulin [1879, 2222, 3666] with some beta globulin [1573]. Several factors influence the degree of the turbidity which develops. Lowering the pH of the buffer, as well as decreasing its ionic strength, increases the turbidity, and thus the sensitivity, of the reaction [1879].

EFFECT OF SERUM PROTEINS. The relation of the serum proteins and thymol turbidity is complex [1431]. Increase of gamma globulin [1879, 2147, 2148], beta globulin [609], lipoproteins migrating with beta globulin [1879], or serum lipids [1879, 2726] enhances the turbidity. Albumin exerts a stabilizing influence [3666] which depends not only upon its concentration but even more on its quality, in that albumin of patients with hepatitis is less stabilizing than albumin of normal persons [2148]. Hepatocellular degeneration thus provides a tendency for turbidity.

EFFECT OF LIPIDS. The depression factor in cholestasis may lessen the turbidity. This is best illustrated by abnormally low turbidity at the height of the obstructive jaundice, which may greatly increase with subsidence of the jaundice. Extraction of lipids with ethyl ether, but not with petrol ether, prevents the turbidity [2726]. Heparin, which diminishes lipemia of the serum, abolishes the thymol turbidity [1334, 3465], as does the addition of Tweens [1879]. The effect of the thymol reagent on the lipids consists of increasing their particle size, and mere serum-lipid elevation without any other alteration in the serum may significantly increase the turbidity. Therefore, the thymol turbidity can be utilized as an oral lipid-tolerance test to evaluate intestinal absorption of fat after administration of standard doses of fat [2644].

FACTORS IN DISEASES. Different factors account for abnormal turbidity in different diseases or different stages of the same disease. In acute

hepatitis, alteration in thymol turbidity parallels changes in the serum lipids, whereas in chronic hepatitis it parallels changes in gamma globulin [1879]. The thymol-turbidity test yields results similar to those of the cephalin-flocculation test except for a more specific elevation in acute and chronic hepatitis, which suggests an influence of gamma globulin or antibody reactions so far not understood.

Method. The main differences in various methods concern the unitage, which depends on the standard curve used. The original units were based on gelatin standards [2147]. The introduction of spectrophotometric readings with barium sulfate standards to replace the original units resulted in the use of two barium chloride solutions [3019] and values differing in various institutions by a factor of two (see Turbidity Standards, earlier in this chapter). The higher values are preferable because they permit better appreciation of the low ranges. Present buffers differ from the original ones [2206]. By lowering the pH from the originally recommended 7.8 to 7.55, the sensitivity has been increased [2240]. This has not necessarily been considered an advantage [2147]. An alcoholic thymol stock solution and stable barbital buffers have been recommended [1569]. The temperature at which the test is run influences the results; it should be kept constant at 25°C.

Technique. REAGENTS.

1. Thymol, USP, recrystallized. Dissolve thymol in 95 per cent alcohol, filter, reprecipitate with cold water, and dry the precipitate in a desiccator with anhydrous calcium chloride for a few days.

2. Barbital buffer. Dissolve 2.06 gm sodium barbital and 2.76 gm barbital in distilled water, with the volume made up to 1.0 liter.

STOCK SOLUTION. Make exactly a 10 per cent solution of thymol in 95 per cent alcohol. Place about 800 ml of the barbital buffer solution in a liter volumetric flask and add 5.0 ml of the 10 per cent alcoholic thymol solution. Dissolve the thin oily layer of thymol by shaking vigorously for 30 to 60 seconds so that no oil droplets are seen in the neck of the flask. Then make up the volume to 1.0 liter. This solution is kept at room temperature and used until it becomes turbid.

PROCEDURE. Just prior to use, dilute 0.5 ml of the 10 per cent alcoholic thymol solution to 10.0 ml with the thymol barbital stock solution in a volumetric flask. Again shake well. Then dilute 0.1 ml serum with 6.0 ml of the finally prepared thymol reagent. Invert the mixture and allow to stand for 30 minutes. Readings are made after this time in a spectrophotometer at a wavelength of 650 or in a colorimeter with a red filter.

Turbidity results are expressed in units by plotting results on the standard turbidity curve.

Results. The normal values depend on the method used. In general 5.0 units appears to be the upper limit of normal, with an average of 3.0 units if the higher standard is used [1875], whereas between 0 and 2 units has been given for the lower standard [2149]. Elevation of thymol turbidity shows good correlation with the morphologically recognizable degree of liver damage [2645].

HEPATITIS. Thymol turbidity is greatly and regularly increased with infectious hepatitis [948, 1427, 1875, 2897, 3184] (Table 22). The eleva-

Table 22 Results of Thymol-turbidity Test in Hepatobiliary Disease

Diagnosis	No. cases	Mean, units	Range, units	% abnormal
Normal.....	347	2.4	0.8- 5.0	2.4
Control patients.....	286	2.2	0.8- 5.6	12.9
Acute hepatitis.....	785	10.2	1.5-37.5	84.0
Cirrhosis with jaundice.....	190	9.6	0.7-33.8	78.3
Cirrhosis without jaundice.....	165	6.7	0.7-25.9	54.4
Extrahepatic biliary obstruction.....	343	4.3	0.8-24.8	26.4
Xanthomatous biliary cirrhosis.....	34	13.4	3.8-40.4	85.7
Hepatic tumor metastases.....	123	2.9	0.5-16.6	26.6
Chronic passive congestion.....	208	4.8	1.0-15.0	47.8
Miscellaneous non-hepatic diseases....	311	35.6

Sources: Method—Maelagen [2147], Shank and Hoagland [3019]; data—Felder *et al.* [990], Mendelsohn and Bodansky [2267], Popper and Schaffner [2640], Ricketts and Wissler [2767], Shay and Harris [3028], Thompson and McCabe [3326], unpublished data [3394], Watson and Hoffbauer [3510].

tion roughly parallels the severity of the disease and persists longer than abnormal cephalin flocculation, so that persistent elevation may characterize chronic low-grade hepatitis [1879]. Elevation of thymol turbidity appears useful in screening blood donors for hepatitis [1026]. Elevated turbidity in apparently healthy people occurs more frequently than increased cephalin flocculation

[948], possibly a sign of the carrier state of serum hepatitis. In toxic hepatitis, particularly when caused by arsenic, the deviation is not so regular and may be absent [2632]. The turbidity is elevated in experimental intoxications [1179]. In acute intrahepatic cholestasis the thymol turbidity is slightly increased or normal.

CIRRHOSIS. In cirrhosis without jaundice slight elevations or normal values are common [1875, 2206, 2763, 3184, 3513, 3554]. It is more often abnormal in cirrhosis with jaundice than in cirrhosis without jaundice. In nutritional or alcoholic cirrhosis the thymol turbidity is not increased or is only slightly increased, whereas in postnecrotic cirrhosis it is very high [1875, 2206]. This is partly caused by the high gamma globulin level in chronic hepatitis, whereas the low levels in alcoholic cirrhosis, despite elevated cephalin flocculation and gamma globulin, may be caused by alteration in serum lipids. In uncomplicated fatty liver, thymol turbidity is usually normal [431, 3700]. In fatty liver with jaundice, the elevation is less frequently seen than an increased cephalin flocculation.

EXTRAHEPATIC BILIARY OBSTRUCTION. In uncomplicated extrahepatic cholestasis, thymol turbidity is normal or slightly increased—up to 7.0 units [2147, 3513]. With protracted obstruction, when fibrosis or secondary bacterial infection occurs, the turbidity rises above 7.0 units, simulating “medical jaundice” [3184].

OTHER DISEASES. The thymol turbidity is increased in many chronic inflammatory diseases, probably as an expression of gamma globulin elevation. These include rheumatoid arthritis, rheumatic fever, lymphopathia venereum, disseminated lupus erythematosus [506, 948, 1875, 2180, 3226], malaria, kala-azar [1875, 2025], and chronic tuberculosis (Table 23). In multiple myeloma it is not usually elevated [1875]. It is elevated in most cases of infectious mononucleosis [417, 1661].

Evaluation. Although the thymol-turbidity test is not a test of hepatic function, it is sensitive for use in screening for hepatic-cell degeneration, but the frequent occurrence of abnormal values in many nonhepatic diseases deprives it of specificity. The test appears particularly useful in the early diagnosis of viral hepatitis, for the recognition of protracted stages and for differentiation of cirrhosis following hepatitis from other types of cirrhosis. Results are usually normal in uncomplicated extrahepatic and intrahepatic cholestasis, in contrast to most forms of primary hepatitis. Since

results are expressed in units, they are better quantitated than results of the cephalin-flocculation test.

Table 23 Diagnostic Significance of Thymol Turbidity

- I. Hepatic diseases associated with increased thymol turbidity
 - A. Hepatitis, especially viral
 - B. Cirrhosis, especially postnecrotic
 - C. Infected and prolonged extrahepatic cholestasis
- II. Extrahepatic conditions associated with increased thymol turbidity
 - A. Hyperlipemia
 - B. Collagen diseases, especially rheumatoid arthritis
 - C. Gastrointestinal disorders
 - D. Inflammatory diseases with elevated level of gamma globulin
 - E. Infectious mononucleosis
 - F. Kala-azar, malaria, and other tropical diseases
- III. Hepatic diseases in which increased thymol turbidity may be the only laboratory clue to diagnosis
 - A. Anicteric hepatitis
 - B. Protracted or convalescent hepatitis
- IV. Diffuse hepatic diseases in which normal thymol turbidity frequently occurs
 - A. Cholestasis (extrahepatic and intrahepatic)
 - B. Cirrhosis with or without jaundice
 - C. Fatty liver
 - D. Toxic hepatitis

Thymol Flocculation

Reading the flocculation which develops within 24 hours after dilution of the serum with the thymol reagent is a modification of the thymol-turbidity test [2416]. The flocculation can be graded arbitrarily by visual inspection or can be expressed as the ratio between the original turbidity and that remaining 18 hours after flocculation [3026]. The results of the thymol-flocculation test do not necessarily parallel those of the thymol-turbidity test, and some consider it to be more specific [2147, 2444]. In other hands the results have been less encouraging [3184]. The test is much less widely used than the thymol-turbidity test.

Dilution-turbidity Test

Dilution of serum with water produces a turbidity that somewhat parallels that of thymol turbidity [832]. Extraction of the serum with ether abolishes the turbidity, as in the case of thymol turbidity. The test is rather simple; globulin has a precipitating effect and albumin a stabilizing effect [853]. The results are similar to

those of the thymol turbidity test [853, 2488], but the incidence of false positive results is supposedly less [32], although the turbidity is less intense. The test has been extensively used in the Orient in screening for kala-azar [3107].

Cadmium Reaction

In the cadmium reaction the turbidity which develops after mixing serum with a cadmium sulfate solution is measured [3665]. The precipitate formed consists primarily of gamma globulin, but if alpha or beta globulin is elevated, the precipitate may contain them. The greater the increase in any individual globulin fraction, the greater is its participation in the reaction. Albumin is precipitated only if reduced, suggesting that a qualitative change of the albumin is responsible for its precipitation. Its stability and simplicity have been listed as the main advantages of the reaction. No significant differences from the cephalin-flocculation and thymol-turbidity tests are noted in the results.

Magnesium Chloride-flocculation Test

One of the oldest flocculation tests is the magnesium chloride test [182]. Gamma globulin is the main precipitating factor; albumin and the other globulins do not flocculate, nor are they said to have an inhibiting function. Results of the test are abnormal in infectious hepatitis and most forms of cirrhosis.

Mercuric Chloride-titration Test

Mercuric chloride in Hayem's solution precipitates with protein when added to serum. This lends itself to either a titration or a flocculation reaction [1293]. The results parallel those of the thymol- and dilution-turbidity tests in the hepatic diseases but not in neoplastic diseases, congestive heart failure, and diabetes mellitus [832]. The results are less specific than those of the cephalin-flocculation and thymol-turbidity tests. It may have some value as a screening test, and the results are normal in multiple myeloma in spite of the gamma globulin elevation [3412].

Colloidal Gold-flocculation Test

Standardized colloidal gold solutions which are routinely utilized for spinal fluid examinations are flocculated by serums of patients with liver disease [1259]. This is an expression of increased gamma globulin [1259], with albumin [1671] and (probably even more) alpha or beta globulins

exerting a protective effect [249]. The buffer concentration and its ionic strength greatly influence the results [2148]. The preparation of the reagent is elaborate, and frequent restandardizations are necessary [2241]. This is the reason why the test is infrequently used in recent years despite its original popularity [2147, 2640, 2740]. The results are similar in principle to those of the cephalin-flocculation test, and a strongly positive reaction is said to almost exclude extrahepatic biliary obstruction [2240]. However, the incidence of abnormal results in nonhepatic diseases with high gamma globulin levels is very high.

Colloidal Red Test

This test has been developed in order to avoid the complications and expenses of the colloidal gold solution by substituting another colloid [853, 2183]. Flocculation results from increased beta and gamma globulin according to some investigators [3663], and from increased gamma globulin alone, according to others [32]. Albumin has an inhibitory effect [32, 461, 3421], and the effect of the depressing factor is noted in cholestasis [461]. Abnormal results occur in many nonhepatic diseases [2198]. In view of the importance of the gamma globulin elevation for the flocculation, the test is less frequently abnormal in acute hepatitis than in cirrhosis [32, 3184].

Other electronegative colloids, such as benzoin, gum gutta, silver or copper ferrocyanide, india ink, and Poirrier blue, have been substituted for colloidal gold or colloidal red with similar results [461].

Takata-Ara Test

The Takata-Ara test deserves special consideration because it was the first flocculation test widely used in liver diseases. It was originally described for use in pleural or spinal fluid in various nonhepatic diseases and then later applied to serum as a test for hepatic disorders.

Many modifications exist [2178], but in principle the test consists of measurement of flocculation in mixtures of serum dilutions with mercuric chloride-sodium carbonate and originally also fuchsin. Either various serial serum dilutions [3383] or serial reagent dilutions [3149] are used. The borderline of flocculation or the number of tubes with flocculation determines the results. The mercuric chloride test more recently developed is actually only a modification of the Takata-Ara test. The evaluation of the flocculation in many of the

modifications is often difficult, because not only the number of tubes but the degree of flocculation in each tube must be taken into account [2178].

Abnormal results are chiefly related to greatly increased gamma globulin in the serum [3663]. Albumin depresses the flocculation somewhat; whether elevation of beta globulin also plays a role is questionable.

The results are abnormal only in the minority of cases with acute hepatitis [3184, 3554] and are not related to the severity of the disease [3554]. This deprives the test of value in the differential diagnosis of jaundice. In transitions into cirrhosis, as well as in fully developed cirrhosis, results are abnormal in a high percentage of cases [2178, 3060, 3184, 3554], the percentage of abnormal results being higher in the posthepatic group than in nutritional cirrhosis. Abnormality has been related to the fibrosing reaction [2801].

Despite early enthusiasm, the test is not specific for hepatic disorders and is not sensitive for the recognition of liver damage [2178, 3184, 3554]. Results are less easily interpreted than those of other flocculation tests. The method is not standardized and is more complicated in its performance. Therefore, the Takata-Ara test is no longer widely used.

Chloranilic acid recently has been suggested as a flocculating agent, with the use of only one tube [598]. Results are similar to those of the Takata-Ara test.

Formol Gel Test

This test is based on the formation of a gel if neutral formalin is added to serum. The reaction had been used widely for many diseases, such as subacute bacterial endocarditis [279]. It indicates gamma globulin elevation above 2.6 gm per 100 ml serum [3421]. With a standardized method it may have some value in hepatic diseases.

COORDINATED USE OF FLOCCULATION TESTS

Although each of the flocculation tests mentioned is dependent on the same main factor, namely, the reduced stability of the serum in the presence of increased gamma globulin, in most of the tests one or more additional factors are involved, especially reduction and alterations of the stabilizing serum proteins, albumin and alpha globulin. The number of possible variations is un-

limited, in that different substances as well as conditions may be used to challenge the stability of the serum colloids. These additional factors are responsible for different results of the individual tests in various clinical conditions. These results often supplement each other, which justifies the performance of several tests in a coordinated system, or flocculation profile. For the performance of this profile any number and type of tests can arbitrarily be used. Several profiles have been proposed [2147, 2149, 2740, 3031, 3136, 3666]. The combined application of cephalin-flocculation, thymol-turbidity, zinc sulfate-turbidity, and gamma globulin-turbidity tests is an example of such a flocculation profile which has been used. This selection is practical, since three of the four tests selected use the same standard curves and differ only in the diluting reagent. The performance of these four tests requires less time and skill than almost any other hepatic test.

For intelligent application of the profile, the factors determining the results of each reaction bear repetition (Table 24). Since the tests do not measure hepatic function, abnormal results are not specific for hepatocellular degeneration. Parallel reduction of both stabilizing proteins albumin and alpha globulin, and not a reciprocal behavior as in nonhepatic disorders [2627], and a qualitative alteration in these proteins [2148] occur almost exclusively as a result of hepatocellular degeneration. Therefore, abnormal cephalin flocculation and thymol turbidity in the absence of gamma globulin elevation point to liver damage, while elevation of gamma globulin and zinc sulfate turbidities with normal cephalin flocculation and thymol turbidity is found in chronic in-

flammatory conditions, in collagen diseases, and in multiple myeloma, as well as in inactive cirrhosis or transition into cirrhosis. When all are elevated in afebrile diseases, hepatic disorders should be considered, and in febrile states a reaction of the reticuloendothelial system is likely [3665]. Differences between thymol turbidity and cephalin flocculation supposedly distinguish the etiology of cirrhosis, in that abnormal thymol turbidity suggests posthepatic cirrhosis, while a normal thymol turbidity in the presence of abnormal cephalin flocculation suggests nutritional cirrhosis.

Table 24 Theoretical Effect of the Presence of Single Pathophysiologic Features on Various Flocculation Tests and Actual Findings in Common Hepatobiliary Diseases

Pathophysiologic feature	Gamma globulin	Zinc sulfate turbidity	Cephalin flocculation	Thymol turbidity
Hepatic-cell degeneration.....	0	1+	3+	3+
Mesenchymal reaction.....	3+	3+	1+	1+
Cholestasis.....	0	2+	1+	1+
Lipemia.....	0	1+	0	2+
Disease				
Acute hepatitis.....	1+	2+	3+	3+
Cirrhosis with jaundice.....	3+	3+	3+	0-3+
Cirrhosis without jaundice.....	2+	2+	1+	0-1+
Transition to cirrhosis.....	3+	3+	1+	1+
Intrahepatic cholestasis.....	0-1+	0	0	0-1+
Extrahepatic biliary obstruction.....	0-1+	0	0	0-1+
Nonhepatic RE stimulation and plasma-cell proliferation....	1-3+	1-3+	0-2+	0-2+

Key: 0, no change; 1+, mild increase; 2+, moderate increase; 3+, severe increase.

Many reactions, some simple and some complex, reflect alterations in serum proteins and their breakdown. Since many of the serum proteins are produced by the hepatic cells, and some probably by reticuloendothelial cells in the liver, the results of these reactions are different in the various hepatic diseases. Some of these methods have been widely applied in the routine diagnosis of liver disease, and some show promise for practical use if methods are simplified and standardized.

Weltmann Serum-coagulation Reaction

When normal human serum is diluted 1:50 and boiled, protein coagulation takes place only if small amounts of calcium salts are added. In some exudative or inflammatory conditions, higher concentrations of calcium chloride than normal are needed, and in primarily fibrosing conditions, lower concentrations suffice. Calcium chloride solutions in various concentrations in 11 tubes are each mixed with 0.1 ml serum and heated for 15 minutes. The number of tubes in which coagulation is found determines the width of the coagulation band. A turbidity reaction, the nephelogram, based on the same principles, has given better quantitative results [3663]. The main responsible factor seems to be alpha globulin, in that the higher its concentration, the smaller the band pointing to exudative conditions [2919]. Beta globulin and albumin also seem to shorten the band, whereas gamma globulin lengthens it, at least in conditions without increased antibody formation. If the gamma globulin elevation is associated with inflammation and increased antibody formation, the band is shortened [3663].

In the hypergammaglobulinemias of hepatic origin, such as cirrhosis or hepatitis and in the

later or infected stages of cholestasis, the band is widened. In acute cholestasis or in tumor metastases it is normal or even shortened [3663]. The combination of the Weltmann serum-coagulation reaction with the cadmium-turbidity test has been recommended as a simple method of determining protein [3664]. The Weltmann reaction may have some practical value in the differentiation of hyperglobulinemias of hepatic origin and those of extrahepatic origin, and it has been recommended for follow-up in cirrhosis [3069, 3445]. However, the test is not of greater practical value than the flocculation tests.

In recent years other heat coagulation-protein reactions have been described, primarily for the diagnosis of cancer [1573], the results of which do not check with the Weltmann coagulation band [1185].

Erythrocyte Sedimentation Rate

The rate of erythrocyte sedimentation depends on several physical and chemical factors, primarily in the blood plasma [2437]. The bile acids seem to exert an inhibitory effect, but the main factor of importance in liver disease is the shape and size of the plasma proteins. Of these, albumin somewhat retards sedimentation, whereas proteins with larger molecules accelerate it. In hyperglobulinemias the sedimentation rate is generally increased, the main accelerating effect being exerted by elevation of alpha globulin and fibrinogen [3032, 3663]. Alpha globulin increase is usually associated with elevation of fibrinogen [2194, 3663].

In hepatic diseases divergent influences appear, and the results are not uniform. In early hepatitis, the sedimentation rate is normal or even reduced, and in the later stages it becomes elevated [1505].

In Weil's disease it is uniformly elevated, as it is in most forms of cirrhosis. In passive congestion it is low [3663]. In obstructive jaundice without infection the results are variable, and in infected forms the rate is usually high. It is also elevated in the presence of tumor metastases. If interfering factors are taken into account, the sedimentation rate may be helpful (1) as an index of persistent activity in convalescent hepatitis; (2) for differentiation of anicteric cirrhosis from chronic passive congestion; (3) in the differential diagnosis of severe jaundice, a low rate indicating parenchymal rather than extrahepatic involvement [2640].

Complement Titer

Complement is one of the serum globulins, possibly in combination with phospholipids [875], which has been considered a product of the reticuloendothelial system. It is reduced in clinical and experimental hepatic injury, while high values are seen in cholestasis, although not with regularity [859, 1658].

Serum Mucoproteins

Physiologic Basis. Serum mucoproteins (serumucoids or glycoproteins) are carbohydrate-protein complexes with relatively little peptide nitrogen and large amounts of polysaccharides in the form of glucosamines and sulfur-containing polymers. The complexes are not clearly defined. The results of quantitative determinations vary, depending upon which moiety, protein or carbohydrate, is measured. The protein moiety appears to be largely α_1 globulin [1273, 2254]. The mucoprotein level is reduced when α globulin is low, as in severe hepatic failure. The origin of the mucoproteins is not established. They seem to originate in part from connective tissue, since high levels are observed in chronic inflammatory conditions such as tuberculosis, in cancer, collagen diseases, and in conditions with excess antibody formation [895, 2989, 3631].

Methods. Most methods are based on the solubility of the mucoproteins in solutions which precipitate other proteins. In a perchloric acid filtrate, in which other proteins are insoluble, mucoproteins can be determined by measurement of the carbohydrate moiety, the amount of tyrosine, or the amounts of protein, either with biuret reagent (see Reagents, under Total Serum Protein, Chap. 32) or simply as the turbidity produced by phosphotungstic acid [1568]. The carbohydrate moiety

of the serum proteins can be estimated by treating paper strips obtained by zone electrophoresis with periodic acid and basic fuchsin (see Paper Electrophoresis, under Fractionation of Serum Proteins, Chap. 32) and measuring the color developed.

Results. Normal serum contains between 80 and 130 mg mucoprotein per 100 ml serum. In hepatic diseases, such as severe hepatitis and cirrhosis, the mucoproteins are low, especially in postnecrotic cirrhosis [1273, 1274, 3631]. The reduction in hepatic disorders involves mainly the protein moiety, while the carbohydrate moiety may remain normal. This suggests a defect in the formation of the protein moiety, probably by the liver. In acute infections complicating hepatic insufficiency, the mucoproteins increase when the α globulin rises. The serum mucoproteins are often elevated in biliary obstruction and regularly elevated in secondary hepatic carcinoma [1274]. C-reactive proteins, which are related to α globulins, are also absent or low in hepatic diseases [2254].

Evaluation. As methods are being standardized, determination of carbohydrate-protein complexes in serum appears promising in the differential diagnosis of liver disease. At present the serum mucoproteins aid in the differentiation of primary hepatic diseases from hepatic tumor metastases and biliary obstruction. They also permit the recognition of carcinoma formation in cirrhosis by the elevation of an otherwise low level.

Prothrombin-complex Activity

Physiologic Basis. Prothrombin, factor VII, and AC-globulin, serum proteins formed by the hepatic cell, are reduced in hepatic-cell degeneration. Many observations concerning prothrombin may therefore apply to other members of the prothrombin complex, also determined by the non-specific methods in widespread use. Other factors, such as deficiency of vitamin K which is needed in formation, influence their blood level [675, 1176, 2677].

Prothrombin time is determined in many diseases to detect coagulation defects; it is also determined during anticoagulant therapy. In liver diseases it is measured mainly for three reasons: (1) to test blood coagulation before surgical procedures or liver biopsy; (2) for control of vitamin K therapy; (3) for evaluation of hepatic function for diagnostic reasons. The method to be used depends upon the purpose

of the test. Whether deficiency of prothrombin or any other protein of hepatic origin is determined is of little consequence in preoperative testing of coagulation. The formation of the other proteins essential in coagulation is usually disturbed by the same hepatic processes which interfere with prothrombin formation. Lack of specificity can thus become an advantage, but to avoid confusion the method applied should be indicated.

Methods. All methods used for determination of prothrombin or members of the prothrombin complex are variations of coagulation-time measurements, made more specific by adding to the blood various factors to identify the missing component.

Howell's prothrombin time, which was named before the role of prothrombin was clearly understood, actually determines two unknowns, thromboplastin and prothrombin. Since deficiency of the former may delay coagulation for minutes, and deficiency of the latter only for seconds, the method practically depends only on thromboplastin, and it is of little use in liver disease.

ONE-STAGE METHOD. In Quick's one-stage method [2678], the plasma-prothrombin time is defined as the minimal time interval for the formation of a grossly visible clot after the addition of excess thromboplastin and calcium. The results depend more on the amount of factor VII and AC-globulin present than on the amount of prothrombin. The result also is influenced by the rate of formation of thrombin from prothrombin and by the rate of the final formation of fibrin. In this conversion, antithrombin plays an ancillary role [3177]. In fibrinogenopenia, fibrinogen has to be added before prothrombin time can be determined. The plasma-prothrombin time as measured by the one-stage method is a result of many factors and, from a practical point of view, is advantageous for preoperative considerations.

Modifications. The one-stage method is the most widely used, and many modifications and variations exist, including micromethods [1700], bedside tests, and dilutions [45, 3390] and variations in thromboplastin [38, 2227]. The recent use of prothrombin time in controlling anticoagulant therapy has further increased the variations in methods used. Commercial thromboplastin from desiccated brain extracts and the use of undiluted plasma are preferable. Modifications of the one-stage method using serum [3176, 3177] or measurement of prothrombin consumption [2680] do not seem to add much in the use of the method in the management of liver disease.

Expression of Results. The coagulation time is determined on the patient's plasma as well as on normal plasma. Results are expressed as prothrombin activity, i.e., as a percentage of normal, from interpolation on a curve originally described by Quick [2678]. This curve is made by plotting the coagulation time against the concentration of prothrombin in serial dilutions of a normal plasma. The hyperbolic shape of the curve is responsible for the fact that slight increases in coagulation time reflect large changes in prothrombin activity, except in the very low ranges [2015]. Dilution of the serum has been recommended to overcome this difficulty, but it introduces new difficulties by diluting other coagulation factors. The actual times do not serve as measurements, nor is the prothrombin time of the unknown expressed as a percentage of the control [38], since this leads to erroneous interpretations in view of the fact that the curve is not a straight line.

TWO-STAGE METHOD. In the two-stage method the amount of prothrombin itself is more definitely established by measuring the amount of thrombin, disregarding its rate of formation [3488]. The results are expressed in units, one unit causing clotting in 1.0 ml of a fibrinogen solution in 15 seconds [3488]. The process of coagulation is subdivided, the conversion of prothrombin to thrombin being completed in a first phase. The thrombin thus formed is permitted to act on a standardized fibrinogen solution. Various modifications exist [1580, 3486]. The results of two-stage methods more specifically reflect hepatic function and clinical severity of hepatic injury [2204, 2207, 2566, 3626, 3710] than do those of the one-stage method. The technical difficulty of the method discourages its wide use as a hepatic test.

DIFFERENTIATION OF MEMBERS OF PROTHROMBIN COMPLEX. Prothrombin, factor VII, and AC-globulin can be separated by performing the simple one-stage test and adding either fresh plasma, aluminum hydroxide, or old serum to the reaction mixture. With fresh plasma all factors are present. Prothrombin and factor VII are removed by aluminum hydroxide treatment, while only factor VII remains in old serum [276].

No attempt will be made to describe any method of prothrombin determination, since the methods commonly applied for control of anticoagulant therapy are usable for hepatic disorders.

Results. ONE-STAGE METHOD. In the one-stage method prothrombin-complex activity depends on the modification used. In general, reduction of the activity below 80 per cent is abnormal, and reduction below 40 per cent may be associated with bleeding. In hepatobiliary diseases, the levels of prothrombin activity are frequently reduced, especially in cirrhosis (Tables 25 and 26). In hepatitis in children, hyperprothrombinemia has been reported [2709]. In acute extrahepatic cholestasis the prothrombin time is normal except when severe hepatic-cell damage is present

[2067, 2082, 2486]. In external biliary fistulas in both man [1593] and animals [1262, 1434], the prothrombin activity is reduced, since bile is necessary to maintain prothrombin activity [2678]. The prothrombin activity in biliary fistula dogs is more sensitive to Dicumarol, and hypoprothrombinemia may be induced by minute doses [1002].

TWO-STAGE METHOD. The two-stage method, with normal values of 240 to 365 units, often detects hypoprothrombinemia in liver disease when the findings of the one-stage test are inconclusive [2204, 3710]. It is better for following the clinical course of liver disease [2207], but abnormal results are found in nonhepatic disorders, and it is insensitive in viral hepatitis.

VARIATIONS OF MEMBERS OF PROTHROMBIN COMPLEX. In acute liver damage of a moderate degree, factor VII is somewhat reduced, and prothrombin is slightly less reduced. In severe hepatic injury factor VII and prothrombin are greatly reduced, and AC-globulin is somewhat decreased. In cirrhosis, all three factors are reduced. A significant reduction of AC-globulin is a sign of poor prognosis. In cholestasis, reduction of AC-globulin does not occur, at least in the early stages [1051]. This has been considered an important differential diagnostic sign.

Response of Prothrombin Time to Vitamin K Administration. In some hepatobiliary disorders, such as prolonged extrahepatic cholestasis with disturbed intestinal absorption of vitamin K, the prothrombin activity is reduced despite unimpaired hepatic ability for prothrombin-complex formation. In contrast, damaged hepatic cells may be unable to form prothrombin complex even with an adequate supply of vitamin K. The response to vitamin K therapy, rather than prothrombin activity, is an indication of hepatic function [451, 2067, 2610], and this is one of the few true hepatic-function tests [2640].

RESULTS. The most commonly accepted criterion for diagnostic purposes entails a return to the normal, or at least a 15 per cent rise within 24 or 48 hours after parenteral administration of 2 to 10 mg synthetic vitamin K salts [78, 2640]. The response is poor in acute hepatitis, especially at the height of the disease, and in cirrhosis. A return to normal after 24 hours and a drop after 48 hours is an abnormal response, as is a gradual ascent to normal only 48 hours after vitamin K administration with low values still present after 24 hours. Therefore, instability of the prothrombin

Table 25 Diagnostic Significance of Prothrombin Activity

- I. Hepatic diseases associated with reduced prothrombin activity
 - A. Cirrhosis *
 - B. Prolonged cholestasis
 - C. Hepatitis *
 - D. External biliary fistulas
- II. Extrahepatic conditions associated with reduced prothrombin activity
 - A. Anticoagulant therapy
 - B. Essential hypoprothrombinemia
 - C. Icterus neonatorum
 - D. Chronic gastrointestinal disorders
- III. Hepatic diseases in which reduced prothrombin activity may be the only laboratory clue to diagnosis
 - A. Prolonged hepatitis, especially toxic
 - B. Cirrhosis without jaundice
- IV. Diffuse hepatic diseases in which normal prothrombin activity frequently occurs
 - A. Any hepatic disease

* Responds poorly to administration of vitamin K.

Table 26 Results of Prothrombin-time Determinations in Hepatobiliary Diseases

Diagnosis	No. cases	Mean, %	Range, %	% abnormal
Normal.....	66	99	95-100	0
Acute hepatitis.....	135	80	10-100	40.8
Cirrhosis.....	229	63	20-100	71.4
Extrahepatic biliary obstruction.....	50	82	41-100	29.2
Xanthomatous biliary cirrhosis.....	6	89	75-100	0

Sources: Method—Quick [2678], Warner *et al.* [3488]; data—Armas-Cruz *et al.* [96], Mann *et al.* [2204], Mann [2207], Quick [2677], Ricketts *et al.* [2761], Ricketts and Wissler [2767], unpublished data [3394].

level after administration of vitamin K is also an indication of hepatic insufficiency.

These irregularities in the response make interpretations difficult and have prevented the widespread use of this test.

VITAMIN K-TOLERANCE TEST. If large amounts of synthetic vitamin K salts are given, hyperprothrombinemia can be produced in normal individuals [3391]; paradoxically, in liver injury, the prothrombin activity drops with these doses [3390]. This fact led to the development of a vitamin K-tolerance test as an allegedly sensitive measure of hepatic function [3390]. This is not a true tolerance test.

Evaluation. Determination of prothrombin-complex activity, even by the simpler one-stage method, is probably the most important laboratory test in the management of the jaundiced patient. It permits evaluation of bleeding tendencies before surgical procedures. The fact that the one-stage method determines not only prothrombin but also factor VII and AC-globulin is an advantage. For the evaluation of hepatocellular degeneration, the prothrombin activity should be an ideal method, but the results are inconstant. The response of the prothrombin activity to vitamin K therapy is no better for diagnostic use, in view of the unpredictable duration of the response. Determination of prothrombin concentration by the elaborate two-stage method more closely measures hepatocellular degeneration, although because of its complexity, this test offers little advantage over simpler procedures. Additional experiences are required to establish the value of the components of the prothrombin complex in the differential diagnosis of jaundice.

Antithrombin

Antithrombin is a coagulation-inhibiting factor bound to serum albumin [2677]. It is activated from a precursor stage, antithrombinogen, by trypsin in the blood derived from the pancreas. The antithrombin titer is increased in pancreatic disorders with increased blood trypsin, such as acute pancreatitis, exacerbation of chronic relapsing pancreatitis, and cancer of the head of the pancreas. It is reduced in pancreatic disorders which destroy pancreatic tissue, such as extensive carcinoma or extensive cystic fibrosis [1591], and in experimental pancreatic destruction [1593]. The chemical nature of antithrombin is not established; it is considered a product of hepatic cells [1593]. The reduction of the antithrombin titer

is said to indicate the extent of liver damage [1593, 3003]. The technique is a modification of the prothrombin-time determination [1591, 2677]; the test is not difficult to perform in laboratories doing coagulation studies.

Insufficient data have been published to confirm the observations of the original authors. The relation of pancreatic and hepatic disorders is complex (see *Relation between the Liver and Exocrine Pancreas*, Chap. 61). Nevertheless, the theoretical basis of the procedure is sound, and the method appears promising. The test may provide an additional criterion of impaired protein synthesis by the hepatic cells. It may assist in the differential diagnosis of jaundice, because an increase would indicate extrahepatic cholestasis from a pancreatic tumor. A normal or even a decreased level is not diagnostic, since it does not exclude extrahepatic cholestasis or even pancreatic tumors.

TESTS RELATED TO PROTEIN CATABOLISM

Total Amino Acids in Blood and Urine

In liver disease, the level of the serum amino acids is elevated, owing either to increased breakdown of tissue proteins in the liver or elsewhere or to reduced utilization of nitrogenous substances by the liver. Altered hepatic function is also reflected in increased amounts of amino acids in the urine.

Serum Amino Acids. Occasional elevations of serum alpha amino nitrogen in hepatic disorders have long been known to occur, but in cirrhosis and hepatitis normal values have been recorded [778, 2093]. In the blood an increase of several individual amino acids, such as methionine [1781], glycine [778], glutamine [3475], and possibly tyrosine [1996], is noted [3475]. Tyrosinemia is found in various types of hepatic failure, in extrahepatic cholestasis, and in nonhepatic disorders, but lack of regularity and difficulties in methods deprive these findings of practical diagnostic significance.

Urinary Amino Acids. The total urinary excretion of amino acids is difficult to determine. It is reported to increase in hepatic diseases [1996], but again without regularity [1781]. Recently described microbiologic and chromatographic techniques permit the demonstration of the spectrum of individual amino acids in the blood and especially in the urine.

MICROBIOLOGIC DETERMINATIONS. Increased excretion of methionine in hepatic disorders was found by several investigators [863, 1781]. The other essential amino acids were generally not altered [2749], although increased urinary tryptophane has been reported [1113]. These changes do not depend upon the diet and as yet have no clinically diagnostic importance or prognostic significance.

CHROMATOGRAPHIC OBSERVATIONS. In fatal hepatocellular degeneration, many amino acids or their metabolites are excreted greatly in excess of normal [3475]. In nonfatal hepatitis or cirrhosis considerably more amino acids than normal are excreted, particularly tyrosine, cystine, and glutamine [772]. This aminoaciduria is also found in experimental animals with nutritional hepatic necrosis [771]. In Wilson's disease [650, 2246, 3398] or Fanconi's syndrome [770], the urinary excretion of most amino acids is especially high.

TYROSINURIA. Tyrosine and leucine crystals have been known to be present in the urine in severe hepatic disorders since Frerichs and Staedeler first demonstrated them in the middle of the nineteenth century. The tyrosine crystals consist of brushlike tufts of fine, colorless or greenish-yellow needles. The identity of the leucine crystals has never been established with certainty.

MILLON TEST. For the demonstration of tyrosine in the urine, chemical methods such as the simple Millon's reaction for the phenol ring are preferable.

Technique for Millon Test. Several methods are described for the preparation of Millon's reagent. The simplest is to dissolve 10 gm Hg in 20 gm fuming nitric acid. This is then diluted with an equal volume of distilled water and allowed to stand 24 hours. The supernatant is decanted and used as such. The urine is heated to boiling in a test tube and a few drops of the Millon's reagent are added. If tyrosine is present, a brick-red precipitate forms. Salicylates and barbiturates form a yellow precipitate, which turns red if excess nitric acid is present.

Tyrosinase Method. More specific quantitative methods employing the enzyme tyrosinase have been described [1996]. The results are comparable to those of the simple qualitative method. Quantitative methods led to the conclusion that tyrosinuria reflects autolysis in the liver and that tyrosinuria in excess of 100 mg per 100 ml is characteristic of massive continued autolysis, as seen in acute liver atrophy. Tyrosinuria may precede hypertyrosinemia. Milder degrees of tyrosinuria

measured by the quantitative method are a fairly sensitive sign of hepatic-cell degeneration and are also found in extrahepatic cholestasis or neoplastic diseases of the liver.

Results. A positive reaction to the Millon test does not necessarily reflect severe hepatic-cell degeneration; it is occasionally encountered in milder cases even without jaundice. Quantitative demonstration of massive tyrosinuria by the more elaborate method is required for confirmation. The appearance of a positive Millon test reaction in the urine when it had previously been negative, or of a strongly positive reaction on the first examination, is a grave prognostic sign.

Evaluation. Aminoaciduria indicates unusual metabolic disorders, such as Fanconi's syndrome or Wilson's disease. Of all the tests demonstrating amino acids in urine or blood, demonstration of tyrosinuria has diagnostic significance in liver disease. Tyrosinuria as simply demonstrated with Millon's reagent is important as an alarm signal to indicate massive hepatic-cell degeneration.

Nonprotein Nitrogen and Urea

PHYSIOLOGIC BASIS. Reduced urea formation and low blood-urea levels are expected in hepatic injury because of defective deamination by the damaged liver. In very severe human hepatic insufficiency such low blood-urea levels have been recorded [2452, 3282], while amino acid levels are very high. This results in an alteration of the normal urea nitrogen/amino acid nitrogen ratio. However, in most instances of severe hepatic disease, the blood-urea level is significantly elevated [3599]. This increase is also reflected in elevation of the nonprotein nitrogen (NPN). The NPN may be even more elevated than urea, because it includes other nitrogenous substances. Therefore, in some hepatic disorders in which the blood urea nitrogen is reduced, or at least not elevated, the NPN is higher than normal [2277, 3282].

The elevation of blood urea and NPN in liver disease is caused by two factors. One is increased protein catabolism, resulting in release of nitrogenous breakdown products into the blood stream, and responsible for the negative nitrogen balance found in liver disease [2638]. The other factor is impairment of renal excretion of urea, caused by renal damage associated with hepatic injury. This results in abnormal glomerular filtration and tubular reabsorption of nitrogenous substances, particularly urea, as indicated by greater reduction

of urea clearance than of creatinine clearance in liver disease [2277].

Renal changes probably are the most important factors in the elevation of urea in liver diseases and outweigh the defect in deamination as far as the blood-urea level is concerned. Therefore, elevation of NPN and urea in the blood is an expression of the renal injury associated with hepatic disorders.

RESULTS. In viral hepatitis, the NPN is not elevated in the acute stage and only rarely so in the chronic form. In contrast, in toxic hepatitis, commonly associated with simultaneous renal injury, high NPN values are frequent. They are also often found in prolonged extrahepatic cholestasis or in the cholestatic phase of cirrhosis. In these conditions cholemia is often associated with uremia, although in the individual case the correlation between NPN elevation and renal injury as seen at necropsy is not clear.

EVALUATION. Determination of NPN is of prognostic value, indicating a serious condition in both primary hepatic injury and extrahepatic cholestasis. In the latter, elevation of the NPN points to a sudden deterioration of hepatic and renal function. The mortality rate in hepatic conditions with an NPN above 70 mg per 100 ml was found to be 78 per cent; when the NPN was below 40 mg per 100 ml, the mortality rate was only 28 per cent [2277].

Uric Acid

The serum-uric acid level may be elevated [409] or reduced in hepatic conditions. Chemical injuries more frequently elevate the level, and renal factors probably play an important role. The uric acid elevation in the absence of elevation of other nitrogenous substances in eclampsia has been associated with liver damage and increased breakdown of nucleoproteins [482].

Amino Acid-tolerance Tests

PHYSIOLOGIC BASIS. The response of the total amino acid level to the administration of amino acid mixtures was one of the early attempts to

evaluate hepatic function. In recent years this has been elaborated by the use of protein hydrolysates of greater purity and by better biochemical techniques. Various amino acid mixtures [943], gelatin [2196], or glycine [778] were administered. Also, individual amino acids have been used in blood or urine.

INTRAVENOUS TESTS WITH AMINO ACID MIXTURES. After administration of modern protein hydrolysates, patients show delayed plasma clearance and decreased urinary excretion of urea, indicating impaired deamination [2093]; the results, however, are erratic [1982, 2093, 3221]. Individual amino acids are adequately metabolized, with the possible exception of methionine [1781].

ORAL TESTS. Oral tolerance tests with proteins or amino acid mixtures have not proved helpful. The urinary excretion of amino acids does not mirror protein intake [1113]. In rabbits with experimental liver damage, the disappearance of injected amino acids from the blood is delayed.

METHIONINE-TOLERANCE TESTS. Studies with methionine also fail to show uniform results. The blood-methionine level after intravenous administration of a test dose returns more slowly to normal in many patients with liver disease than in controls; this is interpreted as an expression of impaired anabolic or catabolic utilization. The urinary sulfate excretion is used to differentiate the anabolic from the catabolic effect [1768]. After oral intake of methionine, its excretion in the urine is increased in some patients with liver disease [114, 3569].

EVALUATION. The disappearance of the amino acids from the blood during a load test does not clearly measure any hepatic function. It may mean utilization for protein synthesis or catabolic deamination to urea or ammonia, or it may merely mean storage in any organ, as has been shown for intravenously administered plasma or albumin [921]. The amount of urinary urea excreted after infusion of amino acids more clearly mirrors a hepatic function, namely, deamination. These tests provide interesting physiologic information but are of little diagnostic value.

TESTS REFERRING TO ENZYMES, CARBOHYDRATES, AND LIPIDS

TESTS BASED ON ENZYMES

The serum enzymes are serum proteins formed mainly by the hepatic cells. They are discussed separately, since their main characteristic is enzymatic action and because their activity rather than their concentration is measured. The diagnostic significance of alterations of their activity depends on the role of the liver as well as that of other organs in the formation, excretion, inhibition, or activation of the enzyme.

Serum Alkaline Phosphatase

Physiologic Basis. Serum alkaline phosphatase is formed normally by osteoblasts, intestinal mucosa, and possibly the liver. In hepatobiliary diseases its activity is increased far more in cholestasis than in hepatocellular degeneration. This is caused by three factors: (1) piling up or regurgitation of phosphatase of extrahepatic origin, particularly in cholestasis; (2) stimulated formation by hepatic cells in the presence of hepatic-cell damage or cholestasis; (3) formation by proliferated ductules, accounting for the very high levels seen in cholestasis and other conditions with ductular proliferation. Therefore, slight elevation in jaundice may be caused by hepatocellular degeneration, whereas greater elevation implies extrahepatic or intrahepatic cholestasis.

Methods. The principle of most of the methods is the incubation of serum with an organic phosphate compound and the subsequent determination of either the liberated phosphate [325, 1754] or other parts of the compound, such as phenol [1756], phenolphthalein [1574], or betanaphthol [2996]. Each of the methods described has certain disadvantages, depending on the stability of the solutions or the optimal enzyme concentration

[2996]. In the United States the method of Bodansky [325], using glycerophosphate, and the method of King and Armstrong [1756], using phenyl phosphate, are the most widely applied.

Technique for Bodansky Method. REAGENTS.

1. Buffered alpha-glycerophosphate substrate. Dissolve 2.15 gm sodium glycerophosphate and 2.12 gm sodium barbital in water and make to 500 ml. Transfer to 1-liter, glass-stoppered bottle and preserve with a 3-cm layer of petrol ether. Keep in refrigerator.
2. Trichloroacetic acid, 10 per cent solution.
3. 2.5 per cent ammonium molybdate solution. Filter before using.
4. Aminonaphthol sulfonic acid reagent. Dissolve 30 gm sodium bisulfite (NaHSO_3) and 1.0 gm sodium sulfite (Na_2SO_3) in water in a 200-ml volumetric flask. Add 0.5 gm amino-2-naphthol-4-sulfonic acid, and make to volume with distilled water. Leave in dark over night. Shake at intervals. Filter and keep in dark bottle.

PROCEDURE. Pipette 0.5 ml serum into each of two tubes. To one, a sample, add 5.0 ml buffered substrate and incubate for exactly 1 hour at 37°C. After 1 hour add 4.5 ml trichloroacetic acid. To the other tube, a blank, add 9.5 ml trichloroacetic acid. Filter both and transfer 3.0 ml filtrate into respectively marked tubes. Add 0.5 ml ammonium molybdate. Shake. Add 0.5 ml aminonaphthol sulfonic acid solution. Add 4.0 ml distilled water. Read at a wavelength of 620.

CALCULATIONS.

- $$\begin{aligned} \text{Sample} \times 2 &= \text{total phosphate in milligrams per 100 ml} \\ \text{Blank} \times 2 &= \text{serum inorganic phosphate in milligrams per 100 ml} \\ \text{Total phosphate minus inorganic phosphate} &= \text{alkaline phosphatase activity in Bodansky units} \end{aligned}$$

Results. The values of alkaline phosphatase are usually expressed as enzyme activity in units per 100 ml serum. These units depend upon the

method used. They vary in normal persons from 1.5 to 4.0 units with the Bodansky method, and from 4.0 to 13.0 units with the King-Armstrong method. In healthy children the values are higher, ranging from 4.0 to 8.0 Bodansky units. In conditions with increased osteoblastic activity, such as Paget's disease, rickets, and primary and secondary bone tumors, the values are high [325]. Starvation lowers the serum alkaline phosphatase while fat ingestion increases it, both in man and experimental animals [3261]. Carbohydrates and proteins are without effect. In passive congestion, mild elevation is common. It rises more with auricular fibrillation [990]. Alkaline phosphatase determinations have been used in animal experiments to evaluate liver damage and biliary obstruction [835, 838, 1551].

HEPATITIS AND CIRRHOSIS. In hepatocellular degeneration alkaline phosphatase activity is usually moderately elevated, ranging between 4 and 12 Bodansky units. The elevation is not well correlated with the degree of hepatic-cell damage [1074, 2645, 3040]. The activity is moderately elevated in viral hepatitis [2709, 2897, 3554], and persistence of this elevation may be an indication of chronicity. In cirrhosis variable results are reported. In the forms without jaundice as a rule the elevation is slight [1314, 3554], and only exceptionally are very high values encountered [2640]. In cirrhosis with jaundice the values are usually considerably higher [3293, 3554]. With severe jaundice the values may rise very high.

CHOLESTASIS. In extrahepatic cholestasis, alkaline phosphatase activity usually rises above 12 Bodansky units (Tables 27 and 28) [1312, 1314, 1464, 3409]. Increase of alkaline phosphatase is thus a reliable sign of cholestasis if other factors are taken into account. In incomplete, infected, or subsiding obstruction, elevation of alkaline phosphatase activity is often out of proportion to the degree of hyperbilirubinemia [3387]. In cholangitis, the activity may be high even in the absence of jaundice. In prolonged obstruction the levels become very high and may reach 50 Bodansky units. The activity is moderately elevated in intrahepatic cholestasis, and levels approaching 50 Bodansky units are rare [27, 3236]. Progressive increase during the course of a disease associated with jaundice speaks against intrahepatic cholestasis.

GRANULOMATOUS PROCESSES. In tuberculous involvement of the liver the activity of the alkaline phosphatase is frequently somewhat elevated.

Table 27 Diagnostic Significance of Serum-alkaline Phosphatase Activity

- I. Hepatic diseases associated with increased serum-alkaline phosphatase activity
 - A. Cholestasis (extrahepatic and intrahepatic) (very high)
 - B. Hepatitis
 - C. Cirrhosis
 - D. Cholangiolitic ("xanthomatous") cirrhosis (very high)
 - E. Primary hepatic carcinoma (very high)
 - F. Metastatic carcinoma
 - G. Biliary fistulas
 - H. Hepatic tuberculosis
- II. Extrahepatic conditions associated with increased serum-alkaline phosphatase activity
 - A. Childhood
 - B. Bone diseases, including carcinoma metastasis
 - C. Gaucher's disease
 - D. Following fat meals
- III. Hepatic diseases in which increased serum-alkaline phosphatase activity may be the only laboratory clue to diagnosis
 - A. Primary and secondary carcinoma
 - B. Cirrhosis
 - C. Incomplete extrahepatic cholestasis, especially with infection
 - D. Intrahepatic cholestasis, cholangiolitis
 - E. Convalescent viral hepatitis
 - F. Cholangitis, even without jaundice
- IV. Diffuse hepatic diseases in which normal serum-alkaline phosphatase activity frequently occurs
 - A. Acute hepatitis
 - B. Fatty liver

Table 28 Results of Serum-alkaline Phosphatase Determinations in Hepatobiliary Diseases

Diagnosis	No. cases	Mean, Bodansky units	Range, Bodansky units	% abnormal	
				Mildly elevated	Markedly elevated
Normal	28	2.6	1.2-5.3	7.1	0
Control patients	164	0	0
Acute hepatitis	546	7.1	2.1-35.6	55.8	10.1
Cirrhosis with jaundice	298	7.5	1.6-35.0	65.1	8.2
Cirrhosis without jaundice	249	5.0	1.5-13.7	55.9	0
Malignant biliary obstruction	267	30.6	3.9-69.8	21.0	76.0
Benign biliary obstruction	225	14.0	3.1-53.1	63.4	32.7
Xanthomatous biliary cirrhosis	34	28.2	9.2-80.4	23.1	76.9
Hepatic tumor metastases	115	9.9	2.1-64.8	49.1	36.9
Chronic passive congestion	150	5.8	1.8-15.0	36.1	2.8

Sources: Method—Bodansky [325]; data—Felder *et al.* [990], Mendelsohn and Bodansky [2267], Popper and Schaffner [2640], Ricketts and Wissler [2767], Shay *et al.* [3029], Thompson and McCabe [3326], unpublished data [3394], Watson and Hoffbauer [3510].

This is rather constant when tuberculous granulomas are predominantly found in the liver. Also in sarcoidosis the levels are frequently moderately elevated. The activity is particularly high if intrahepatic cholestasis is caused by tuberculosis [595] or sarcoidosis.

HEPATIC FAILURE. Rarely, in severe hepatic failure the alkaline phosphatase activity is low even if the condition would ordinarily increase activity. Patients with prolonged extrahepatic biliary obstruction usually die with high alkaline phosphatase activity, although in some instances severe hepatic failure causes a terminal drop.

TUMORS. In primary hepatic carcinoma, serum-alkaline phosphatase activity is frequently increased, becoming exceedingly high when one of the large intrahepatic bile ducts is obstructed. A moderate elevation to about 10 Bodansky units commonly occurs in carcinoma metastases to the liver in the absence of jaundice [438, 441, 1314, 2640], and this diagnosis should be suspected if Bromsulphalein retention is also increased [3031]. Increased phosphatase activity was found in 90 per cent of patients with advanced hepatic metastases and in 44 per cent in metastases without hepatomegaly [2267]. If in addition bone metastases are present, the phosphatase activity may reach levels over 30 Bodansky units. In hepatic abscesses, elevation of alkaline phosphatase activity occurs in the absence of jaundice merely as an indication of a space-occupying lesion.

Evaluation. Determination of the serum activity of alkaline phosphatase is one of the most useful hepatic tests despite its lack of specificity and its unsettled physiologic basis.

Elevated serum-alkaline phosphatase activity has different implications, depending upon the presence or absence of jaundice. In the icteric patient, a moderate alkaline phosphatase elevation speaks for either hepatocellular degeneration or lesser degrees of cholestasis and therefore has little diagnostic value. Elevation above 15 Bodansky units indicates extrahepatic or intrahepatic cholestasis. It therefore is one of the most valuable laboratory signs for the differentiation of extrahepatic, or "surgical," obstruction from primary hepatitis in conjunction with other tests, if possibilities such as cholangitis, cirrhosis, or primary hepatic carcinoma are taken into account. Absence of a great increase has more diagnostic significance than its presence. Discrepancies between greatly increased serum-alkaline phosphatase activity and a slight elevation of the serum bilirubin

speaking for incomplete and infected extrahepatic biliary obstruction and cholangitis. In the absence of significant jaundice, moderate elevation of alkaline phosphatase activity speaks for either a space-occupying lesion, such as carcinoma metastases or hepatic abscess, or for a granulomatous process, such as tuberculosis or sarcoidosis. Greatly increased alkaline phosphatase activity in the absence of jaundice or in mild jaundice speaks either for cirrhosis, primary hepatic carcinoma, or secondary carcinoma with bone metastases. Under all circumstances bone diseases have to be taken into account as a cause of increased alkaline phosphatase activity. Terminal drop of alkaline phosphatase activity, observed rarely, indicates severe hepatic failure.

Serum Esterase

Physiologic Basis. Serum esterase preferentially splits short-chain fatty acid esters, including acetylcholine, and is therefore also called serum cholinesterase or pseudocholinesterase, in contrast to the true cholinesterase of the nervous system and red cells. The serum-esterase level is related to hepatic function. Serum esterase supposedly originates from the liver, because it is found there in abundant amounts. Since the serum esterase generally parallels the serum albumin, the serum-esterase level has been also considered an index of the protein-forming ability of the liver.

Methods. The original methods used acetylcholine as a substrate and determined the carbon dioxide liberated [2094, 2922]. Subsequently, phenyl benzoate was used, with determination of the phenol liberated [1212]. In recent years manometric methods with benzoylcholine chloride as a substrate have been used [3618]. Electrometric measurements of pH changes resulting from the liberation of acetic acid from acetylcholine provided a simple method applicable for routine clinical use [2283]. Since this entails alteration of the enzyme activity throughout the incubation because of the change in pH, a method was described based on colorimetric determination of the disappearance of acetylcholine bromide in buffered solutions [1571].

Technique [1571]. REAGENTS.

1. Barbitol buffer. Dissolve 10.3 gm sodium barbitol in 300 ml distilled water and slowly add 60 ml *N* hydrochloric acid. Barbitol crystals appear. Add 5.3 gm anhydrous sodium carbonate. Warm and stir until the crystals dissolve. Add water to 500 ml. Keep in refrigerator.

2. Salt mixture. Dissolve 4.2 gm anhydrous magnesium chloride, or 910 gm $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, and 0.2 gm potassium chloride in water and make volume to 100 ml.

3. Acetylcholine bromide—11.3 per cent solution. Recrystallize salt by dissolving 100 gm in 600 ml ethyl alcohol. Filter and place in refrigerator for 12 hours. Collect the crystals, wash with ether, and vacuum dry over sodium hydroxide pellets. Keep frozen.

4. Acetylcholine-buffer-salt mixture. Mix eight volumes of barbital buffer and one volume each of acetylcholine solution and salt mixture immediately before using.

5. Hydroxylamine hydrochloride—14 per cent solution. May be kept in refrigerator for 1 week.

6. Sodium hydroxide—14 per cent solution.

7. Alkaline hydroxylamine. Mix equal volumes of the last two solutions just before using.

8. Ferric chloride solution. Dissolve 10.0 gm ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in 1.0 liter 0.002 *N* hydrochloric acid. A tenfold-concentrated stock solution may be kept more conveniently.

9. Hydrochloric acid solution. Make a 5.0 per cent solution by diluting 50 ml concentrated hydrochloric acid up to 1.0 liter with distilled water.

PROCEDURE. Transfer 0.2 ml serum or oxalated or citrated plasma to a test tube. Into a second test tube place 0.2 ml water as a control. To each tube add exactly 0.2 ml acetylcholine-buffer-salt mixture previously warmed to 37°C. Incubate 1 hour at 37°C. Add 2.0 ml alkaline hydroxylamine. Wait at least 1 minute, then add 6.0 ml hydrochloric acid solution. Stopper the tubes and invert three times. Transfer 0.5 ml from each test tube into separate tubes and to each of these add 10.0 ml ferric chloride. Invert as above and centrifuge for 5 minutes. Read supernatant at a wavelength of 540. Make a reagent blank by adding 10.0 ml ferric chloride solution to 0.5 ml hydrochloric acid solution and set reading with this to 100. Interpolate readings on standard reference curve prepared by using 2.2 ml acetylcholine bromide solution containing 20 to 100 micromoles, without incubation or addition of salt or buffer. Results are expressed as micromoles per milliliter serum per hour.

Results. The values obtained depend upon the method used. They are expressed as micromoles per milliliter serum per hour with the colorimetric method [1571] or phenyl benzoate method [1212]; as cubic millimeters of carbon dioxide per hour with the manometric method [2922, 3618]; as change in pH in the electrometric method [2283]. The results do not vary in the same person, but the differences between normal persons or patients with similar types of hepatic disease are great.

In general with all methods a significant reduc-

tion in the values indicates hepatic injury (Table 29). Low normal values may represent a reduction for a given patient if serial determinations

Table 29 Diagnostic Significance of Serum-esterase Activity

- I. Hepatic diseases associated with decreased serum-esterase activity
 - A. Cirrhosis
 - B. Hepatitis
 - C. Protracted extrahepatic cholestasis
 - D. Metastatic carcinoma
- II. Extrahepatic conditions associated with decreased serum-esterase activity
 - A. Exposure to difluorophosphate (DFP) or tetraethyl pyrophosphate (TEPP)
 - B. Malnutrition and infections (not consistently)
- III. Hepatic disease in which decreased serum-esterase activity may be the only laboratory clue to diagnosis
 - A. Cirrhosis
- IV. Diffuse hepatic disease in which normal serum-esterase activity frequently occurs
 - A. Early extrahepatic cholestasis

are available [2897]. In acute hepatitis the values are reduced to low normal or abnormal values. The reduction is greater in patients with severe liver damage. The level often rises to normal before other tests yield normal results [3618]. In cirrhosis, the reduction is consistently greater. In biliary obstruction, especially when caused by carcinoma or when complicated by infection, reduction is also noted [3092]. The levels drop following biliary tract surgery, the maximum depression occurring 3 to 4 days postoperatively, with return to normal by the tenth day in uncomplicated cases [3123]. Low levels are also seen in hepatic tumor metastases, in passive congestion of the liver, in malnutrition, in debilitating diseases, in anemias, and in acute infections [3443].

Evaluation. Serum-esterase determinations are a valuable indication of the status of the hepatic cells, reduction of activity paralleling the degree of hepatocellular damage. In view of the constancy of the level in any single individual, the test has value in follow-up and prognosis. It is of little use in the differential diagnosis of jaundice or in the diagnosis of liver disease because of low values in biliary obstructions and diseases not primarily hepatic.

Serum Amylase

Serum-amylase or -lipase elevation points to pancreatic involvement. Reduction in hepatic dis-

eases has been reported [1258] but is not widely used as a hepatic test.

TESTS BASED ON CARBOHYDRATE METABOLISM

The central role of the liver in carbohydrate metabolism and glycogen storage makes tests concerning carbohydrate metabolism valuable tools. Historically, these tests were utilized very early in the evaluation of hepatic function, but the influence of many other factors, such as the carbohydrate and fat content of the diet, insulin, and pituitary or adrenal hormones, makes such an evaluation difficult.

Blood Glucose

The rapid drop of the blood-sugar level in hepatectomized dogs [2202] and reported defects in glycogenolysis or gluconeogenesis in experimental liver damage suggest that the blood-sugar level decreases in hepatic insufficiency. On the other hand, stress reactions that occur in hepatic damage, and disturbed carbohydrate metabolism, particularly in fatty liver [3042], tend to result in hyperglycemia and glycosuria typical of diabetes mellitus. This led to the term "hepatogenic diabetes," indicating a condition in which the damaged liver fails to respond normally to the normal amount of circulating insulin [1937, 3139]. As a result of the conflicting tendencies, the blood-sugar level in hepatic diseases varies. In fatty livers such as those produced by alcohol [1935] and occasionally also in other types of hepatic injury [3139], it is high and associated with glycosuria. In severe human hepatic failure or coma, the blood-sugar level is usually normal or even high [2387].

Liver disease is generally listed as a cause of spontaneous hypoglycemia. It has been reported in exceptional instances of fatty liver and cirrhosis [2261], in primary hepatic carcinoma [3325], and in infectious and toxic [3169] hepatitis. However, hypoglycemia is rare in any liver disease, and if present it is usually not severe and not correlated with hepatic function. It may serve as an alarm signal in severe hepatic insufficiency [2500].

Glucose-tolerance Test

The liver stores various carbohydrates such as glucose, levulose, or galactose in the form of glycogen and serves as the source of blood sugar. The level of homeostatic regulation of the blood sugar

by the liver depends upon its functional state. The blood-sugar level at which glycogen deposition occurs is much higher in liver damage than in normal persons [3139]. Therefore, the response of the blood-sugar level to the administration of glucose reflects the function of the liver if the many other factors which influence the blood-sugar level are taken into account.

RESULTS. Many variations of the glucose-tolerance test have been applied to hepatic diseases. The results are altered in infectious hepatitis and even more in chronic liver disease [2371, 2706, 3139]. In general, a sustained rise in the blood-sugar level is noted in the first and second hours of the test, followed by a hypoglycemic phase. In other instances the tolerance curve, after oral glucose intake, is flat or rises rapidly followed by a rapid return to normal. Glycosuria does not necessarily mirror this diabetic curve. The therapeutic use of intravenous glucose in liver damage leads to glycosuria more readily than normal. The tolerance curve has been ascribed diagnostic significance in differentiating primary hepatic disease from metastatic carcinoma and biliary obstruction in which the curve is normal or, if abnormally elevated, is not sustained; this, however, has not been confirmed [2508, 3397].

BLOOD-SUGAR RESPONSE TO HORMONE ADMINISTRATION. The responses of the blood sugar to various hormones are tests designed to measure the ability of the liver to release glycogen as blood sugar, thus measuring either the size of the glycogen depot or the efficiency of glycogenolysis. Earlier experimental studies indicated that epinephrine failed to induce hyperglycemia after hepatectomy or hepatic artery ligation [3139]. Subsequent results of clinical tests [1770] suggest that the lack of the blood-sugar response to epinephrine is an indication of hepatic insufficiency [1770], but results are erratic under both normal and abnormal circumstances [59, 1494]. The pancreatic hyperglycemic factor, glucagon, has been used in place of epinephrine or in combination with it [3413]. The response to insulin is of little value in measuring hepatic function [3461].

Levulose-tolerance Test

PHYSIOLOGIC BASIS. Levulose, or fructose, is transformed to glycogen only in the liver [661] (see Carbohydrate Metabolism, Chap. 5), and the portal blood is almost completely cleared of fructose after a single passage through the liver. Fructose is transformed to glycogen at a more rapid

rate than glucose. The blood-sugar level rises less, and less sugar appears in the urine following intravenous administration of fructose as compared with glucose [3535], although the renal threshold for fructose is very low. In comparison with glucose, hormonal and dietary factors are less significant and therefore alterations of the fructose metabolism appear to be more specifically related to hepatic function. Historically, the levulose-tolerance test was one of the first procedures for the measurement of hepatic function.

METHODS. The urinary excretion of fructose following the oral intake of 100 gm was originally measured. The low renal threshold leads to excretion of fructose in normal persons as well as in many patients with liver diseases. Consequently, the urinary test for fructose has been replaced by the determination of blood sugars following a test dose. In the earlier methods glucose and fructose were determined together by reduction tests. A rise of the total blood sugar of more than 30 mg per 100 ml or over 125 mg per 100 ml following the intake of 50 gm fructose is abnormal. More recently, the serum-fructose level itself has been determined. After oral administration of 50 gm fructose, a rise over 15 mg per 100 ml beyond the fasting level, which should not exceed 8.0 mg per 100 ml, or a delayed fall in the level is considered evidence of hepatocellular dysfunction. The curve has been simplified, reducing the number of venapunctures necessary [2740].

RESULTS. Abnormal results occur in cirrhosis more frequently than in acute hepatitis, and in both of these groups far more frequently than in extrahepatic cholestasis or carcinoma metastases; results are too erratic for routine use, however.

Galactose-tolerance Test

Physiologic Basis. Galactose is the most quickly absorbed hexose [661]. It is rapidly transformed to glucose, mainly in the liver, with the help of a specific enzyme system [2777, 3351]. This system is deficient in spontaneous galactosemia with galactosuria, which is a rare inborn error of metabolism (see Carbohydrate Metabolism, Chap. 5). The ability of the liver to store administered galactose as glycogen depends on the capacity of this enzyme system, as well as on the glycogenesis from glucose in the liver. Both are probably altered in hepatocellular injury. The transformation of galactose is less influenced than that of glucose by insulin excess or deficiency [1973, 2821], but

it is possibly under the influence of other hormonal factors [3455].

Methods. **URINARY EXCRETION.** Originally, impaired glycogenesis was measured in the galactose-tolerance test by determination of urinary galactose excretion following the ingestion of 40 gm galactose. Galactose has a very low renal threshold, but the excretion depends upon the rate of urinary flow [2202]. Under normal circumstances almost all the galactose ingested is converted to glycogen and the urine contains less than 3.0 gm. Recording the time of excretion has been recommended, in addition to the simultaneous administration of water, to eliminate the factor of renal concentration [1005, 3554]. In the absence of spontaneous glycosuria, simple determination of reducing substances in the urine suffices. Otherwise, the glucose has to be removed first by yeast fermentation. To overcome impaired intestinal absorption of galactose in cirrhosis [51], deficiency diseases [189], and old age, intravenous administration is recommended [175, 1755], usually 0.5 gm per kilogram of body weight.

DETERMINATION OF GALACTOSE IN BLOOD. Galactose can also be determined in the blood rather than through its urinary excretion. Originally, this was done as a simple blood-sugar curve, but now glucose is first removed by yeast [175, 1755]. After oral administration the blood-galactose level normally does not rise above 30 mg per 100 ml [53, 2270], although values up to 80 mg have been reported [2147]. The galactose index, representing the sum of the blood-galactose values at $\frac{1}{2}$, 1, $1\frac{1}{2}$, and 2 hours after the test dose, was recommended as a more reliable criterion. This normally should not exceed 160. The results are well reproducible in the same person [2270].

GALACTOSE CLEARANCE. In recent years the blood clearance after intravenous administration has been preferred [175, 1755]. In normal persons complete clearance after 75 minutes is the rule. Blood clearance has been used with Bromsulphalein and benzoic acid in a single intravenous test dose [3709]. To take into account variations of blood and tissue space volume and the shape of the tolerance curve, a formula for galactose time [158] or for a galactose-removal constant was developed [620]. The latter is based on the kinetics of the disappearance of galactose from the blood [814] and is determined as the galactose removed from the blood per minute. It is based on two blood specimens obtained 15 and 45 minutes after intravenous galactose administration.

The galactose-removal constant appears to be the simplest of the intravenous clearance methods. It shows good correlation with other hepatic tests.

Technique for Galactose-removal Constant. RE-AGENTS.

1. 50 per cent galactose solution [1977]. After the solution has been tested for sterility, it should be stored at 37°C in an incubator to prevent crystallization.

2. A 20 per cent suspension of fresh baker's yeast washed seven times.

3. Protein-precipitating solution consisting of equal amounts of 10 per cent sodium tungstate and two-thirds N sulfuric acid.

4. Benedict's quantitative blood-sugar reagent.

COLLECTION OF SAMPLE. The test is usually performed about 2 hours after the patient has breakfast. First, blood is drawn to furnish a blank for the non-galactose, nonfermenting copper-reducing substances. Thereafter, galactose solution corresponding to 0.5 gm per kg is injected intravenously in 2 to 3 minutes. Blood samples are drawn from the opposite arm at 15 and 45 minutes after the injection and placed in oxalated tubes.

DETERMINATION OF GALACTOSE. Determination of blood galactose is based on Benedict's method for blood sugar, after removal of glucose by fermentation.

To 2.0 ml whole oxalated blood add 12 ml 20 per cent suspension of fresh baker's yeast. Fermentation is complete in 10 minutes. Then add 6.0 ml protein-precipitating mixture to the ferment. The remainder of the procedure, i.e., heating, cooling, colorimetry, follows Benedict's method. Galactose standards (concentrations of 10 or 20 mg per 100 ml) are employed instead of glucose. Recovery experiments to exclude galactose fermentation by the yeast should be performed frequently.

CALCULATION OF THE GALACTOSE-REMOVAL CONSTANT (GRC).

$$GRC = 7.6 (\log \text{ concentration at 15 min} - \log \text{ concentration at 45 min})$$

Normal values range from 4.2 to 9.5, while in liver disease values are usually below 4.0.

Results. The simple oral urinary test reflects the presence of severe diffuse hepatic-cell damage, particularly in acute hepatitis. Normal values are frequently seen in milder hepatitis even with the more elaborate methods. Cirrhosis is usually characterized by delayed excretion [3554]. The intravenous clearance methods appear better for the recognition of liver damage [3040]. In early extrahepatic biliary obstruction, normal results are found—this has been the chief advantage of the test (Table 30). In prolonged or infected obstruc-

tion, the results are abnormal. Abnormalities are also encountered in chronic passive congestion or tumor metastases. These findings result in a great

Table 30 Results of Galactose-tolerance Tests in Hepatobiliary Diseases

Diagnosis	No. cases	% abnormal
Normal.....	162	0
Control patients.....	38	9.5
Acute hepatitis.....	316	64.8
Cirrhosis.....	243	45.4
Extrahepatic biliary obstruction....	301	25.0
Hepatic tumor metastases.....	19	10.0
Chronic passive congestion.....	10	36.8

Sources: Method—Coleher *et al.* [620], MacLagen [2147], Sherlock [3040]; data—Popper and Schaffner [2640], unpublished data [3394].

overlapping, with a high percentage of abnormal values in all hepatobiliary diseases (Table 31). The test has been considered less valuable than other hepatic tests [760].

Table 31 Diagnostic Significance of Galactose Tolerance

- I. Hepatic diseases associated with reduced galactose tolerance
 - A. Hepatitis
 - B. Active cirrhosis, especially with jaundice
 - C. Protracted extrahepatic cholestasis
- II. Extrahepatic conditions associated with reduced galactose tolerance
 - A. Hyperthyroidism
 - B. Galactosemia
- III. Hepatic disease in which reduced galactose tolerance may be the only laboratory clue to diagnosis
 - A. Toxic hepatitis
- IV. Diffuse hepatic diseases in which normal galactose tolerance frequently occurs
 - A. Cirrhosis
 - B. Hepatitis
 - C. Early extrahepatic cholestasis

The galactose-tolerance test seems to be useful in thyrotoxicosis; after oral administration of galactose, a diminished tolerance was demonstrated [54, 1996, 2944]. Since this was not found on intravenous administration [53, 158], increased absorption caused by a sympathomimetic effect in hyperthyroidism was assumed to account for the abnormal galactose tolerance after oral admin-

istration. Some investigators considered liver damage to be an important factor [1996, 2150].

In animal experiments the galactose tolerance has been used for the recognition of hepatocellular degeneration [1758].

Evaluation. The galactose-tolerance tests offer little advantage in the differential diagnosis of jaundice beyond that obtained by simpler methods. For the establishment of the degree of hepatic-cell damage, galactose clearance after intravenous administration, the galactose-removal constant, can be recommended as a confirmatory method. Despite its theoretical soundness and early popularity the test is not frequently used.

TESTS CONCERNING CARBOHYDRATE INTERMEDIATES

Serum Citrate. Determination of serum levels of citrate, an intermediary in the Krebs cycle, had been described as a test of hepatic function. The liver supposedly contains citric acid dehydrogenase which reduces the serum-citric acid level. In clinical as well as experimental hepatocellular degeneration, the serum levels are increased [1664, 3089, 3554]. The level is abnormal in a high percentage of patients with hepatitis, less so in cirrhosis, and infrequently so in extrahepatic cholestasis. The degree of abnormality is only poorly correlated with the degree of hepatocellular degeneration, and hypercitricemia occurs frequently in secondary hepatic carcinoma, chronic passive congestion, nonhepatic gastrointestinal diseases, diabetes mellitus, and uremia. Nevertheless, the test may have some value, since a simplified method has been developed recently inviting investigation of its practicality [954]. The response to the intravenous administration of citric acid parallels the results of determinations of fasting citrate levels [3089].

Serum Pyruvate. Serum levels of pyruvate, an intermediary in carbohydrate breakdown, are elevated not only in thiamine deficiency, thyrotoxicosis, beriberi, and after severe exercise, but also consistently in hepatic coma [62], especially after glucose is given [63]. In liver disease without coma this elevation is absent even after glucose administration. The determination of pyruvate levels in serum is therefore not a diagnostic hepatic test.

Serum Lactate. Lactate in serum results from glycogen breakdown in muscle. When it reaches the liver, it is rebuilt to glycogen and eventually

returned to muscle as glucose—the Cori cycle. In liver diseases, the serum-lactate level is often elevated above 20 mg per 100 ml. The irregularity of the results, as well as the finding of similar elevations in many other conditions, such as cardiac failure, deprives the determination of any clinical significance.

Lactic Acid-tolerance Test. The response of the serum-lactate level to lactic acid administration has been used as a test of hepatic function [3131]. The theoretical basis of the test is not clear, since administered racemic lactic acid is not taken up by the livers of normal experimental animals [1607]. On the contrary, if glucose is available as a glycogen precursor, lactic acid is produced by the liver. In animals that are starved or fat fed, lactic acid is taken up by the liver.

After intravenous injection of sodium *d*-lactate to patients with liver diseases and to experimental animals with liver damage, the lactate level of the blood rises higher and decreases later than normal [612, 3131]. Diagnostic significance was attributed to this behavior, especially in the differentiation of surgical and medical jaundice [3131]. The results of earlier experiments were thought to be invalid because of the use of racemic sodium lactate. The practical diagnostic value of the test is no greater than that of the galactose-tolerance test, whereas the preparation of the reagents and the laboratory procedures are far more elaborate [1996].

TESTS BASED ON FAT METABOLISM

The liver is the main source of the serum phospholipids and serum cholesterol, and it influences the level of other blood lipids. In addition to quantitative changes of lipids in liver diseases, qualitative alterations occur and have recently been demonstrated with newer physical methods, such as ultracentrifugal lipid fractionation [3377] and zone electrophoresis [867]. As yet only quantitative changes determined chemically are used in the routine study of liver diseases.

Total Serum Lipids

Physiologic Basis. The total serum-lipid value is the sum of the individual fractions, of which cholesterol and phospholipids are the most important. Both these substances and the total lipids show similar trends in liver diseases, in that in severe hepatocellular degeneration the levels are low, while cholestasis tends to increase them.

Method. Simple turbidimetric methods are available for estimation of total serum lipids [1877, 2181]. The turbidity developing following dilution of the serum with phenol does not exactly mirror the total lipid concentration. Therefore, another turbidimetric method was developed, giving good correlation with the total lipid concentration [1571].

Technique. REAGENTS.

1. Bloor's mixture—mix one volume of ethyl ether with three volumes of absolute alcohol.

2. Dioxane.

3. Sulfuric acid solution, 4.0 per cent by volume.

PROCEDURE. Into a 10-ml calibrated test tube or graduated centrifuge tube, pipette 0.5 ml serum and add 9.5 ml Bloor's mixture. Place in a water bath at 50 to 60°C for ½ hour or longer. Cool and add Bloor's mixture to the 10-ml mark. Invert the tube and filter or centrifuge. Transfer 1.0 ml of the filtrate to another test tube and evaporate to dryness in boiling water. Add 1.5 ml dioxane and place in boiling water for 1 minute. Allow to cool to room temperature and add 5.0 ml of the sulfuric acid solution. Do not shake the tube. After ½ hour at room temperature, read in a spectrophotometer or colorimeter at a wavelength of 650 or with a red filter using water as a blank. If turbidity is too great, a smaller aliquot of the alcohol ether extract can be used.

STANDARDIZATION. For the standard curve, determine the total lipids in different serums gravimetrically by extracting 5.0 ml serum with 95 ml Bloor's mixture. After incubation at 50 to 60°C for 1 hour, make up the volume to 100 ml with Bloor's mixture and filter. Then evaporate 80 ml of the filtrate to dryness, extract the lipids with petroleum ether, then filter, wash, and evaporate in a weighing bottle. Calculate the total lipids in milligrams per 100 ml. Aliquots of 1.0 ml of the same Bloor extract are treated as above in the turbidimetric method, and the percentage of transmission or optical density is plotted against the corresponding value obtained gravimetrically.

Results. The total lipid concentration in serum determined by turbidimetry ranges from 300 to 800 mg per 100 ml. In acute hepatitis and acute extrahepatic biliary obstruction it is elevated. In chronic intrahepatic or extrahepatic cholestasis, the values are very high. They are extremely high in cholangiolitic cirrhosis [1652, 3256] and in some instances of cholestasis [310] and alcoholic fatty cirrhosis [3318]. Low values are found in some instances of severe hepatic failure associated with cirrhosis. However, changes of the total concentration do not occur with sufficient regularity in liver diseases to offer diagnostic aid. The total fatty acids follow the total lipid values [37].

Serum Neutral Fat

Serum neutral fat in liver diseases generally follows the total lipids, the chief exception being cholangiolitic or xanthomatous biliary cirrhosis, in which its concentration is either low [3318] or only very slightly elevated [27]. Neutral fat is usually increased in the serum in obstructive jaundice or hepatitis, subsiding with relief of obstruction or recovery from the disease [2195]. Its determination has no diagnostic value in liver disease.

Serum Phospholipid

Physiologic Basis. The serum phospholipids are entirely or almost entirely formed in the liver. Therefore, in hepatocellular degeneration, their serum level tends to drop, although this may be obscured by a tendency to increase parallel with free cholesterol. In cholestasis the serum-phospholipid level increases as a result of regurgitation or of increased formation in the liver, or of both.

Methods. The serum-phospholipid values are obtained by determining the phosphorus content [1025] of lipid extracts [3684] or of acetone precipitates to provide lipid phosphorus levels. Arbitrarily, these values are multiplied by 25 to express the phospholipid levels expressed as lecithin. More complicated methods determine individual phospholipids such as lecithin, cephalin, or sphingomyelin [3082, 3318]. In the serum of patients with fatty livers the ratio of choline-containing phospholipids—lecithin and sphingomyelin—to non-choline-containing phospholipids—cephalin—varies, but no significant differences consistently occur in liver diseases [36]. Therefore, for diagnostic purposes, the determination of total lipid phosphorus suffices.

Results. Normally the serum contains 8.0 to 12.5 mg lipid phosphorus per 100 ml, with an average of 9.5. In acute hepatitis the phospholipid values show a much wider range than normal (Table 32). They are usually low early and rise in convalescence [37, 1652, 2195, 3318]. They are low in severe hepatic failure, although occasionally the level rises when the free cholesterol increases parallel with a decrease in cholesterol esters. In cirrhosis with jaundice a similar wide range is encountered with a tendency for low values, predominantly in patients with severe hepatic failure. High values are found if cholestasis is a factor [2195, 3256]. In cirrhosis without jaundice, in which hepatic failure and cholestasis are not significant, the range is narrower.

Very high values are found with greater frequency in malignant extrahepatic biliary obstruction than in benign obstruction [37, 310, 1652].

Table 32 Results of Serum-phospholipid Determinations in Hepatobiliary Diseases

Diagnosis	No. cases	Mean, mg %	Range, mg %	% abnormal	
				Low	High
Normal.....	25	9.5	7.8- 12.9	12.0	4.0
Acute hepatitis.....	50	11.9	2.8- 47.8	13.6	29.6
Cirrhosis with jaundice.....	104	9.6	2.2- 83.0	46.2	17.3
Cirrhosis without jaundice.....	49	9.5	4.0- 14.1	26.5	18.4
Malignant biliary obstruction.....	33	22.9	6.1- 49.5	6.1	84.8
Benign biliary obstruction.....	22	11.6	3.1- 53.1	18.2	36.3
Xanthomatus biliary cirrhosis.....	4	60.2	13.1-154.8	0	100.0

Sources: Method—Fiske and Subbarow [1025]; data—Balfour [147], unpublished data [3394].

Relief of obstruction returns the values to normal [1652, 2195, 2278]. In rats with ligated common bile ducts, the phospholipid levels are high without parallel increase in liver phospholipid [3537]. In intrahepatic cholestasis or cholangiolitis with or without xanthomatosis, the phospholipid level is very high [27].

Evaluation. Since serum phospholipids tend to be reduced in hepatocellular degeneration and increased in cholestasis, the phospholipid determination offers an ancillary method for the recognition of cholestasis. However, for the clinical differential diagnosis of jaundice, the rather elaborate procedure can not be recommended, since occasionally high values are found in acute hepatitis, and normal values in early obstruction.

Serum Cholesterol

Physiologic Basis. The liver is an important source of the serum cholesterol. In severe hepatocellular degeneration, serum cholesterol tends to drop, while in cholestasis it tends to rise. Only a small part of the rise can be explained by regurgitation of biliary cholesterol into the blood. Increased formation of cholesterol and elevation of serum bile acids, which apparently increase the cholesterol-binding ability of the serum proteins, also account for some of the cholesterol elevation. Therefore, it does not necessarily mirror the degree and duration of cholestasis. The relation of diet

to the cholesterol level with regard to the pathogenesis of atherosclerosis is much debated and is beyond the scope of this book.

Cholesterol in the blood is in the form of either free alcohol or a fatty acid ester. The liver is the main factor in regulating the esterification processes, even that portion which occurs in serum. Therefore, in liver damage the relative amount of esterified cholesterol is reduced. This reduction occurs more readily with high total cholesterol levels.

Methods. Total cholesterol is determined either by the colorimetric measurement of the green color developed in the presence of acetic anhydride and concentrated sulfuric acid, the Lieberman-Burchard reaction, or by gravimetric or colorimetric [2954] determination of free cholesterol precipitated with digitonin. The colorimetric method has several drawbacks, such as temperature sensitivity, lack of specificity, and differences in color intensity between free and esterified cholesterol [1526, 3543]. The digitonin method used gravimetrically is cumbersome and does not lend itself to routine use. Combination of digitonin precipitation with well-controlled colorimetry and saponification, as exemplified by the Schoenheimer-Sperry method [2954], seems to be an acceptable compromise. This procedure is time-consuming, but it gives useful and reliable results, in contrast to simpler colorimetric methods. Many variations have been published which recommend turbidimetric assay [1681] or determination without saponification [2617]. The following method is almost as accurate as the widely used Schoenheimer-Sperry method and is somewhat less troublesome [542, 2355].

Technique. REAGENTS, ALL ANALYTICAL REAGENT GRADE.

1. Acetone-alcohol. Mix equal quantities of acetone and 95 per cent alcohol.
2. Digitonin. Dissolve one part in 200 parts of 50 per cent alcohol—replace every 30 days.
3. Ethyl ether, redistilled.
4. Acetic acid, glacial.
5. Acetic acid, 10.0 per cent solution.
6. Potassium hydroxide, 33 per cent by weight, prepared by dissolving 10.0 gm potassium hydroxide in 20 ml water. Prepare every 30 days.
7. Hexane, redistilled, or petroleum ether, or Skellysolve B.
8. Standard cholesterol solution. Dissolve 100 mg pure cholesterol in chloroform and make up to 100 ml.
9. Chloroform
10. Acetic anhydride and sulfuric acid. Add 1.0 ml

concentrated sulfuric acid to 9.0 ml acetic anhydride cooled in an ice bath. Prepare fresh just before using.

APPARATUS.

1. Centrifuge tubes, pipettes, or syringes for 0.5, 1.0, 2.0, 3.0, and 7.0 ml.
2. Two blocks of aluminum, provided with 50-watt cartridge heaters and thermostats set for 35°C and 60°C and drilled to hold $\frac{5}{8}$ -in. test tubes and 15-ml centrifuge tubes. Water baths set at the same temperatures may be used.

PROCEDURE.

1. Preparation of filtrate. Place 0.7 ml serum in 15-ml centrifuge tube and add rapidly from a syringe 9.6 ml acetone-alcohol. Stopper with clean cork and let stand 10 minutes at 60°C. Centrifuge 10 minutes at 2,000 to 2,500 rpm. Transfer exactly 3.0 ml clear filtrate to each of two 10-ml centrifuge tubes.
2. Precipitation of free cholesterol. To one centrifuge tube containing filtrate add 1.0 ml digitonin solution and mix by shaking. Place tube in an incubator for 3 hours at 37°C. Centrifuge 10 minutes at 2,500 rpm. Carefully decant the supernatant liquid, wash once with 3.0 ml ethyl ether, centrifuge, and decant again. Evaporate the excess ether with a stream of filtered air at 60°C. Dissolve the precipitate in 0.5 ml glacial acetic acid. Stopper tightly with cork and keep at 60°C for 20 minutes to ensure complete solution. Cool to room temperature. Add 3.0 ml chloroform and save for color development along with total cholesterol.
3. Determination of total cholesterol. Evaporate the contents of the other centrifuge tube containing 3.0 ml filtrate at 60°C to 1.5 ml with an air stream directed with a capillary tube. For multiple determinations, a distributing manifold is advantageous. Add 0.1 ml 33 per cent potassium hydroxide and mix thoroughly. Allow saponification to proceed at 60°C for 30 minutes, using air stream as before. Volume should now be 0.5 ml. If the volume is reduced below 0.5 ml, make up with 95 per cent alcohol. Neutralize with slight excess of acetic acid by adding 0.5 ml 10 per cent acetic acid. Add exactly 4.0 ml hexane from a 5-ml syringe with enough force to mix thoroughly. Mix by drawing up hexane into syringe and discharging it into solution several times. Rinse syringe with hexane between specimens. Immediately stopper tightly with cork. Centrifuge 5 minutes. With a syringe, transfer 2.0 ml of the supernatant hexane layer to a test tube. Evaporate to dryness at 60°C with the aid of a stream of air. Add 0.5 ml glacial acetic acid, then 3.0 ml chloroform. Stopper tightly.
4. Color development. Prepare a standard solution of 0.2 mg cholesterol in 3.0 ml chloroform and 0.5 ml glacial acetic acid. Place the standard and the two tubes containing the cholesterol digitonide and the total saponified cholesterol at 35°C. When they have reached this temperature, in about 10 minutes, add to each rapidly with a syringe 1.0 ml chilled acetic anhydride sulfuric acid mixture, the latter being at a

temperature of 15°C or less. Note time that reagent is added. Immediately restopper and at the end of exactly 8 minutes at 35°C, transfer tubes to an ice bath at -5°C; allow to cool for 5 minutes. Read at a wavelength of 625, using chloroform as a blank. Readings should be made at any time between 20 and 25 minutes after addition of color reagent.

Results: Total Serum Cholesterol. The serum normally contains between 130 and 250 mg cholesterol per 100 ml, with a mean of about 180 and an occasional unexplained value below or above this range in an otherwise healthy person [1737, 3543]. Sex has no influence. The level rises with age above thirty years [1737], while in infants and children the values are not significantly lower than in young adults [1514]. In normal persons the level is constant on serial examinations; in diseases variations are common [2355]. Total cholesterol in whole blood is lower than in serum, because the red cells contain few cholesterol esters. Results obtained in oxalated or citrated plasma also differ from those in serum [3543].

The total cholesterol level is high in obesity, the last trimester of pregnancy, nephrosis, diabetes, familial hypercholesteremia with or without xanthomatosis, hypothyroidism, and after ACTH treatment. It is lower than normal in malnutrition, fever, hyperthyroidism, anemia, and infections in general [3543] (Tables 33 and 34).

CHOLESTASIS. The cholesterol level is raised above 300 mg per 100 ml serum in intrahepatic

Table 33 Diagnostic Significance of Elevated Total-serum-cholesterol Level

- I. Hepatic diseases associated with elevated total-serum-cholesterol level
 - A. Extrahepatic cholestasis
 - B. Cholangiolitic (xanthomatous) cirrhosis
- II. Extrahepatic conditions associated with elevated total-serum-cholesterol level
 - A. Obesity
 - B. Pregnancy
 - C. Nephrosis, nephritis
 - D. Hypothyroidism
 - E. Diabetes mellitus
 - F. Xanthomatosis
 - G. Familial hypercholesteremia
- III. Hepatic diseases in which elevated total-serum-cholesterol level may be the only laboratory clue to diagnosis
 - A. Cholangiolitic (xanthomatous) cirrhosis
 - B. Prolonged cholestasis, especially with infection
- IV. Diffuse hepatic diseases in which normal total serum-cholesterol level frequently occurs
 - A. Hepatitis
 - B. Cirrhosis

and extrahepatic cholestasis. In the extrahepatic form, the level depends to some extent on the duration of the obstruction but not on its degree,

Table 34 Diagnostic Significance of Reduced Total-serum-cholesterol Level

- I. Hepatic diseases associated with reduced total-serum-cholesterol level
 - A. Hepatic coma of any etiology
 - B. Hepatitis
 - C. Cirrhosis
- II. Extrahepatic conditions associated with reduced total-serum-cholesterol level
 - A. Infections
 - B. Anemia
 - C. Malnutrition
 - D. Hyperthyroidism
 - E. Acute pancreatitis
- III. Hepatic disease in which reduced total-serum-cholesterol level may be the only laboratory clue to diagnosis
 - A. Cirrhosis without jaundice

and high levels are seen in incomplete obstruction [37, 310, 1652, 2195, 3318] (Table 35). Relief of obstruction reduces the total serum-cholesterol level [2195, 2278]. In intrahepatic cholestasis, including arsenical hepatitis, the cholesterol levels

Table 35 Results of Total-serum-cholesterol Determinations in Hepatobiliary Diseases

Diagnosis	No. cases	Mean, mg %	Range, mg %	% abnormal
Normal.....	54	237	136-325	17.7
Acute hepatitis...	139	218	56-1,000	16.5
Cirrhosis with jaundice.....	296	187	45-450	7.1
Cirrhosis without jaundice.....	147	204	77-415	7.5
Malignant biliary obstruction....	73	395	100-1,950	50.0
Benign biliary obstruction....	67	262	97-600	19.2
Xanthomatous biliary cirrhosis.	15	1,079	363-2,540	100.0
Hepatic tumor, metastases.....	20	175	69-291	0
Chronic passive congestion.....	136	185	33.0

Sources: Method—Schoenheimer and Sperry [2954]; data—Felder *et al.* [990], Hoffbauer *et al.* [1521], Kydd and Mann [1890], Mendelsohn and Bodansky [2267], Ricketts *et al.* [2761], Ricketts and Wissler [2767], Shay *et al.* [3029], Thompson and McCabe [3326], unpublished data [3394], Watson and Hoffbauer [3510].

may be excessively high parallel with increases in other lipid fractions. In experimental ligation of the common duct high levels are observed.

HEPATOCELLULAR DAMAGE. In severe hepatocellular degeneration, such as fulminant viral hepatitis, the total cholesterol level may be very low [37, 1652, 2195, 3318]. In acute hepatitis the cholesterol level is elevated only if protracted cholestasis is present [37, 1129, 2195]. In most forms of cirrhosis, the total serum-cholesterol level is normal or low [3256] except in biliary cirrhosis, where it may rise to over 1,000 mg per 100 ml serum, or if a cholestatic component is present, in which case levels of 300 to 400 mg per 10 ml are seen.

Results: Cholesterol Esters. The cholesterol esters are usually reported as a percentage of the total cholesterol, rather than in absolute figures. This percentage is normally between 60 and 75 per cent of the total cholesterol regardless of its absolute value [1652, 2195, 3318]. Some investigators consider values between 50 and 60 per cent also normal [2420].

The reduction of this ratio has been considered the lipid alteration most characteristically and most consistently found in liver diseases [1652, 2195, 3318, 3548], although low values are seen as a result of malnutrition alone (Table 36).

Table 36 Diagnostic Significance of Cholesterol/Ester Ratio

- I. Hepatic diseases associated with reduced cholesterol/ester ratio
 - A. Cholestasis
 - B. Cirrhosis
 - C. Hepatitis
 - D. Fatty liver
- II. Extrahepatic conditions associated with reduced cholesterol/ester ratio
 - A. Infection
 - B. Malnutrition
- III. Hepatic diseases in which reduced cholesterol/ester ratio may be the only laboratory clue to diagnosis
 - A. Anicteric hepatitis
 - B. Cirrhosis without jaundice
- IV. Diffuse hepatic diseases in which normal cholesterol/ester ratio frequently occurs
 - A. Hepatitis
 - B. Cirrhosis
 - C. Fatty liver

Values below 20 per cent are most likely the result of hepatocellular degeneration.

With equal degrees of liver damage, the reduction of the cholesterol ester ratio is greater in patients with high total cholesterol. In hypercholesteremia with normal liver function a normal

ester ratio is found [2571], but in hypercholesteremia with even slight liver damage the ratio drops. Therefore, in cholestasis usually associated with hypercholesteremia, reduction of the cholesterol ester ratio without other abnormalities suggests relatively mild liver damage [1760] (Table 37). In hepatocellular jaundice on the other hand,

Table 37 Results of Cholesterol/Ester Ratio (Per Cent of Total Cholesterol Esterified) Determinations in Hepatobiliary Diseases

Diagnosis	No. cases	Mean, %	Range, %	% abnormal
Normal.....	170	71.2	41.7-81.8	3.9
Acute hepatitis....	527	42.3	9.0-64.0	67.6
Cirrhosis with jaundice.....	366	52.1	10.0-78.0	39.5
Cirrhosis without jaundice.....	137	64.2	38.0-81.0	11.9
Malignant biliary obstruction.....	227	38.8	8.0-61.0	75.0
Benign biliary obstruction.....	126	55.0	15.0-74.0	28.2
Xanthomatous biliary cirrhosis...	15	44.5	18.0-72.5	53.9
Hepatic tumor metastases.....	48	65.0	49.0-72.0	56.3
Chronic passive congestion.....	138	60.2	31.0-70.0	38.6

Sources: Method—Schoenheimer and Sperry [2954]; data—Hoffbauer *et al.* [1521], Kydd and Mann [1890], Popper and Schaffner [2640], Ricketts *et al.* [2761], unpublished data [3394].

in which the cholesterol level is usually low, more severe hepatic-cell degeneration is needed to reduce the cholesterol ester ratio significantly. In severe hepatitis, especially the fulminant and fatal forms, the cholesterol ester ratio is very low [37, 2195]. In moderately severe hepatitis the ratio is often reduced [1129]. In general the ratio is inversely proportional to the serum-bilirubin level [3704].

In cirrhosis with jaundice, the cholesterol ester ratio is usually low [3256]. In cirrhosis without jaundice normal values are frequent. In tumor metastases and passive congestion depression of the ratio is frequent.

In experimental injuries produced by carbon tetrachloride, toluylenediamine, or partial hepatectomy, the cholesterol esters are reduced.

Evaluation. The elevation of the total cholesterol level is a valuable sign of cholestasis, extrahepatic or intrahepatic in nature, although the elevation does not necessarily measure the degree of cholestasis. Reduction of the total cholesterol level indicates severe hepatocellular degeneration, but it also occurs in malnutrition. Reduction of the cholesterol ester ratio is a good indicator of hepatocellular degeneration. Milder degrees of damage reduce the ratio more readily in extrahepatic biliary obstruction than in hepatitis or cirrhosis. The ratio has no value in the differential diagnosis of jaundice but is a useful guide in following the clinical course. The complicated technique, especially of the ester partition, is a serious drawback to the widespread application of the partition.

Cholesterol Esterification

Incubation of normal human serum reduces the concentration of free cholesterol. This reduction is less in liver diseases.

Ratios of Serum-lipid Fractions

In addition to the cholesterol ester ratio, the relationship between phospholipids and cholesterol has received much attention. The ratio between phospholipids and total cholesterol behaves erratically in liver diseases and has little diagnostic significance [2195]. The ratio is rather high in all instances of hepatic hyperlipemia, such as cholangiolitic cirrhosis with xanthomas (see "Xanthomatous Form" of Cholangiolitic Cirrhosis, under Cholangiolitis and Pericholangiolitis, Chap. 46), explaining the lack of atherosclerosis in such conditions [27, 2195].

The phospholipids are found in a fairly fixed ratio to the free cholesterol [37, 1876]. This explains the increase of the phospholipid in hepatitis without severe hepatocellular degeneration, when the absolute amount of free cholesterol rises because of reduction of the esters. Only if the disease is severe do the phospholipids drop with the total cholesterol.

Fecal Fat

The increase of fecal fat in various hepatic diseases was originally considered to be the result of impaired absorption of fatty acids in the absence of bile acids. This can not be demonstrated with tagged fatty acids. Neutral fat as well as fatty acids are excessively excreted in the absence of bile acids [2953]. The determination of fecal fatty acids, therefore, and the ratio of free fatty acids

to neutral fat in the feces are not reliable indicators of hepatic function.

Lipid-tolerance Tests

An altered response to the intake of fats in liver diseases reflects poor intestinal absorption in ob-

structive jaundice or hepatitis. This can be demonstrated by absence of the normal increase in thymol turbidity after administration of butter [2644]. Similarly, cholesterol administration to dogs with Eck fistulas leads to a greater rise in serum cholesterol than normal [138].

Many of the earlier theories of bile pigment metabolism are now challenged. This does not necessarily influence the clinical interpretation of changes in the blood or urine levels of the pigments. Therefore, the physiologic basis is reviewed dogmatically, and reference should be made to the arguments in the previous chapters.

SERUM BILIRUBIN

Physiologic Basis. The serum bilirubin is bile pigment formed from the breakdown of hemoglobin in the reticuloendothelial system and excreted by the liver into the bile. An increase in the serum concentration of this pigment is responsible for jaundice, regardless of its cause. Attempts have been made to differentiate types of bilirubin based upon diazo color reactions before and after treatment with alcohol, urea, or caffeine (the van den Bergh reaction). Direct-reacting bilirubin, which gives the diazo reaction before treatment, is said to differ biologically from indirect-reacting bilirubin, which gives the diazo reaction only after treatment. The direct-reacting portion is thought to have passed through the hepatic cells or Kupffer cells and to have returned directly to the blood or to have been regurgitated into the blood from the biliary passages. The indirect-reacting portion has not been taken up by hepatic cells or Kupffer cells. The partition methods and the designation of the fractions vary as a result of many observations and theories about bile pigment metabolism. Although the identity of direct-reacting bilirubin, also called prompt-reacting or 1-minute bilirubin, is still somewhat unsettled, its determination is of practical importance.

Serum bilirubin can be determined either by

estimation of the color of the serum (the icterus index) or by means of the diazo reaction of van den Bergh.

Icterus Index

The icterus index is determined by comparing the color of the serum with bichromate standards either directly or after acetone precipitation. Acetone precipitation avoids error due to hemolysis but may cause absorption of protein to the precipitate. Results after acetone precipitation correlate better with quantitative measurements of specific color reactions [3210]. Yellow colors not from bilirubin but from carotene, Atabrine, or other pigments interfere with the determinations. If this source of error is kept in mind, the icterus index is a simple method for grading the degree of jaundice and for following the course of disease. Spectrophotometric readings on whole blood at two different wavelengths compared to a standard bilirubin solution have been suggested to overcome many of the objections to the icterus index determinations [3049].

RESULTS. The normal values lie between three and eight units. Visible jaundice is present if the levels rise above 15 units. Various formulas have been devised to convert these units to milligrams of serum bilirubin [1664], and complex comparison tables have been published [1996]. The relation of icterus index to bilirubin is approximately 10:1 at lower levels and is less accurate at higher levels. In severe jaundice, values up to 200 units have been observed, but the degree of increase does not necessarily parallel the total bilirubin levels. Simultaneous determinations of both total serum bilirubin and icterus index have been claimed to offer diagnostic advantages [926]

Total Serum Bilirubin Based on the Diazo Reaction

Bilirubin gives a red color reaction [3408] with diazotized sulfanilic acid. Part of the serum bilirubin does so only after treatment with various substances. Originally alcohol was used, but this leads to bilirubin absorption on the precipitated protein and to errors of up to 40 per cent [1256]. Diazotization before precipitation is a means of overcoming this difficulty [1757]. Newer methods use dilute ethyl or methyl alcohol [2192] and dilution of serum to avoid precipitation. This principle is feasible with the use of the spectrophotometer, permitting determination of very faint colors. Protein-precipitation methods have been described that do not absorb bilirubin. Micromethods have been used following quantitative oxidation of bilirubin [1953]. In view of common development of turbidities with any of the alcohol precipitation methods, the use of caffeine is preferable [1638].

Technique. REAGENTS.

1. Diazo reagent. Solution A. Dissolve 0.25 gm sulfanilic acid, C.P., in 3.75 ml concentrated hydrochloric acid. Add water to 250 ml and mix.

Solution B. Dissolve 0.25 gm sodium nitrite, C.P., in 50 ml distilled water. Prepare fresh every 3 days.

2. Buffered caffeine solution. Dissolve 120 gm urea, C.P., 25 gm caffeine, U.S.P., in 300 ml distilled water at 60°C. Cool and add water to make 500 ml.

3. Blank solution. Dissolve 15 ml concentrated hydrochloric acid in water and make up to 1,000 ml.

PROCEDURE. Prepare the diazo reagent freshly before using by diluting 0.3 ml solution B to 10.0 ml with solution A. Add 2.0 ml diazo reagent to 8.0 ml caffeine buffer solution. Add 2.0 ml of this mixture to 0.2 ml serum. Make to 5.0 ml with water and read at a wavelength of 530 after ½ hour. For the blank, add 2.0 ml blank solution to 0.2 ml serum and make to 5.0 ml with water. Standard curves are prepared using crystalline bilirubin [2192]. For the determination of the bilirubin levels in jaundice, the serum dilutions used permit adequate readings on the galvanometer scale. When normal and low levels are expected and when small differences are of diagnostic importance, the serum dilution should be halved (0.4:5.0).

Results. The normal values vary between 0.25 and 1.03 mg per 100 ml serum [3502], with a mean of 0.62 to 0.25 [3708]. Some consider levels above 0.8 mg as elevated [44]. Physiologic variations in the bilirubin level do not raise it above the normal range, and the level generally remains constant. For diagnostic purposes, values above

1.2 mg per 100 ml are considered elevated. Repeated bilirubin determinations are of greater practical value than a single determination. Aggravation or improvement is often reflected in the bilirubin level, which thus becomes one of the most important yardsticks of prognosis in general.

Abnormally low values are found in secondary anemias caused by iron deficiency, tumors, or renal diseases, as well as in primary refractory anemias. Values above 1.2 mg are seen in newborn infants, and the level often rises in the first few days of life up to 10.0 mg per 100 ml in icterus neonatorum.

JAUNDICE. The elevation which occurs in any type of jaundice depends on such factors as the rate of blood formation and destruction, and the renal excretion of bilirubin, in addition to the disease present. Therefore, single bilirubin determinations have no diagnostic value in the differentiation of types of jaundice or clinical disease entities. They are of value when used as a screening test or as an index of the severity of any hepatobiliary or hematologic disorder. Occasionally in severe hepatocellular degeneration in cirrhosis or fulminant hepatitis, hyperbilirubinemia is absent, perhaps because hemoglobin breakdown is altered. Also in mild viral hepatitis, the degree of hepatic-cell damage is poorly reflected in the bilirubin level, as exemplified by anicteric hepatitis [1801, 2897]. In congestive heart failure the serum-bilirubin level is frequently elevated [990].

EXTRAHEPATIC BILIARY OBSTRUCTION. Intermittent or ball-valve obstruction of the common or hepatic bile duct is characterized by fluctuating bilirubin levels. Complete biliary obstruction is usually associated with a constant high level, which reflects the balance of bilirubin formation and urinary excretion. Secondary severe hepatic-cell damage complicating extrahepatic biliary obstruction produces a sudden elevation and is therefore an alarm signal [943, 3185]. This occurs in purulent infections, such as cholangitis or pyelophlebitis, complicating extrahepatic biliary obstruction. Persistent elevation after surgical relief of obstruction indicates either severe liver damage or hepatic metastases.

EXPERIMENTAL ANIMALS. The serum-bilirubin level in dogs is normally lower than in man, not exceeding 0.2 mg per 100 ml. This low level possibly results from a lower renal threshold, since dogs normally excrete bilirubin in the urine. In liver damage, the increase is less striking than

in man [1234]. The level in rats is similar to that in man [1377].

Bilirubin-tolerance Test

The clearance of exogenous bilirubin from plasma in the absence of jaundice has been considered a sensitive method for testing the function of the liver, assuming that this bilirubin is excreted largely by the liver and kidney and not taken up by the reticuloendothelial system. It can also be thought of as a test of the ability of the Kupffer cells to take up bilirubin and transmit it to the hepatic cells. Abnormal results, therefore, indicate either a Kupffer cell-hepatic-cell block or hepatic-cell damage.

In the practical application, 1.0 mg commercial bilirubin per kilogram body weight in an alkaline medium is slowly injected intravenously. Blood samples are drawn before and 5 minutes and 4 hours after the injection. Bilirubin retention is estimated by comparing a mixture of plasma and acetone with a bichromate standard. The pre-injection readings are subtracted from the reading in the 5-minute sample. This serves as the basis for comparison with the 4-hour sample to calculate the percentage retention.

Retention above 6 per cent indicates impaired hepatic function. Calculation of a clearance, the velocity constant of excretion, permits a theoretically more sound measurement of the excretory function of the liver. Mild degrees of liver damage, as in chronic hepatitis, cirrhosis, or congestive failure, can be recognized by this method. The main drawback to the test is the difficulty in obtaining purified and standardized bilirubin solutions.

The response of the plasma-bilirubin level to the intravenous injection of nicotinic acid is an endogenous bilirubin-tolerance test based on the same principle as is the exogenous test. Nicotinic acid induces hyperbilirubinemia, which subsides more slowly than normal in hepatic damage [3176].

Qualitative van den Bergh Reaction

The performance of the diazo reaction both in untreated serum and after addition of alcohol or caffeine is the basis of the classic van den Bergh reaction. It is based on the assumption that the direct-reacting portion has passed through the liver, whereas the indirect-reacting portion has not. The color reaction designated as indirect actually reflects the sum of both direct- and indirect-reacting bilirubin. The direct reaction is

normally negative, while the indirect reaction is faintly positive [3408].

In jaundice from cholestasis or hepatic-cell degeneration, the direct reaction is positive, whereas in uncomplicated hemolytic jaundice it is negative even with a significant indirect reaction. If the serum diazo reagent mixture is kept for more than 10 minutes, a negative reaction may become positive and has been designated as a "delayed reaction." A slight positive direct reaction may deepen and has been termed a "biphasic reaction." The subsequently developing color results from indirect-reacting bilirubin converted to direct-reacting by the acid of the diazo reagent [858]. If one waits long enough all of it becomes direct-reacting.

The qualitative reaction is useful for the separation of hemolytic jaundice from other types of jaundice, but it has been replaced by quantitative measurements of direct-reacting bilirubin as part of the total bilirubin determination.

Determination of Direct- or Prompt-reacting Bilirubin

The direct- or prompt-reacting bilirubin as originally defined by the qualitative van den Bergh reaction is a vague entity quantitatively, particularly since its chemical identity is uncertain. No chemical basis exists for evaluating the several quantitative methods for determination of direct bilirubin, and the selection is primarily based on the clinical value. One principle of partition depends upon the assumption that indirect bilirubin is chloroform- [1443, 3010] or ether-soluble [3416] in contrast to direct-reacting bilirubin, although this has been challenged [858]. The measurement of the color which develops within the first minute of diazotization is recommended as a means of determining prompt-reacting bilirubin [858, 3502], since the color which develops subsequently is indirect-reacting bilirubin converted to direct. The use of 1-minute values is based on curves of rates of diazotization [3502], despite certain theoretical objections [1786]. For most purposes, the serum should be diluted less in the 1-minute determination than in the total bilirubin determination, since the low values are of greatest importance.

Technique. REAGENTS.

1. Diazo reagents A and B, as in total serum bilirubin. Prepare before using by diluting 0.3 ml solution B to 10.0 ml with solution A.
2. Blank solution, as in total serum bilirubin.

PROCEDURE. To 0.5 ml serum add 2.2 ml distilled water and 0.3 ml diazo reagent. For the blank, 0.5 ml blank solution is substituted for diazo reagent. Readings are made at a wavelength of 530 exactly 1 minute after the addition of the diazo reagent. Standard curves are the same as those for the total serum bilirubin.

Results. Under normal circumstances small amounts of prompt-reacting bilirubin are found in serum, not exceeding 0.25 mg per 100 ml, with a mean of 0.11 to 0.05 [3708]. With almost all spectrophotometers available the galvanometer readings for normal values of prompt-reacting bilirubin are in the range of 90 to 98 per cent transmission and are therefore inaccurate. Therefore such values are reliable only insofar as they differentiate normal from abnormal. Elevations above normal are significant mainly in relation to the total bilirubin level.

In the presence of visible jaundice, the level of prompt-reacting bilirubin is useful only in differentiating hemolytic jaundice from other forms. In hemolytic jaundice in the absence of liver damage, the prompt-reacting bilirubin is normal or less than 20 per cent of the total bilirubin [1256, 3708]. The level of prompt-reacting bilirubin is of no value in differentiating the types of jaundice with impairment of bile flow, i.e., jaundice with cholestasis from jaundice with hepatic-cell degeneration. The ratio of the prompt-reacting to total bilirubin in jaundice resulting from impairment of bile flow depends primarily on the degree of jaundice. It is rather low with any type of jaundice if the bilirubin levels are below 3.0 mg per 100 ml, while above this level it rises to 40 to 60 per cent [2905, 2910, 3708].

In initial and defervescent stages of jaundice with impairment of bile flow, the prompt-reacting fraction rises before and may return to normal after the total bilirubin level has done so [482, 858, 2416]. In this sense, the prompt-reacting bilirubin is more sensitive than the total bilirubin in screening for early or slight hepatic damage. In acute gallbladder colic the prompt-reacting bilirubin may rise within 24 hours and return quickly to normal, while the total bilirubin is hardly altered [2909]. In the convalescent stage of hepatitis, other laboratory signs as well as clinical symptoms of activity are often still present when the prompt-reacting fraction is elevated, even though the total bilirubin has returned to normal. On the other hand, persistent elevation of the total

bilirubin with a normal prompt-reacting fraction is usually associated not with clinical or laboratory evidence of hepatic damage but rather with an isolated disturbance of the bilirubin metabolism—either overproduction or elevated threshold.

Evaluation of Serum-bilirubin Determinations. The total serum-bilirubin determination has little diagnostic value but is a very good procedure for following the course of a disease associated with jaundice. It does not necessarily reflect the degree of hepatic injury, since jaundice may be absent even in instances with severe liver damage. The determination of the direct-reacting, prompt-reacting, or 1-minute bilirubin is a valuable screening procedure. When there is no manifest jaundice, it assists in the recognition of hemolytic or overproduction jaundice, in which it is not significantly elevated. In early jaundice or in defervescent stages, elevation of the 1-minute bilirubin suggests liver damage, while in the presence of acute upper abdominal pain it suggests biliary colic, both often with normal total bilirubin levels.

Serum Biliverdin

Biliverdin is an intermediate between hemoglobin and bilirubin. Intact liver function and adequate nutrition are required for the transformation of biliverdin to bilirubin [1945]. Biliverdin also results from oxidation of bilirubin, especially after stagnation in the liver and biliary tract. Under these circumstances, biliverdin appears in the blood, probably by regurgitation [1066, 1544], and is responsible for the green discoloration of the skin. The intradermal injection of a small amount of 0.1 per cent potassium ferricyanide solution produces a blue-green discoloration in the presence of a bivalent iron-containing pigment which has been considered iron-biliverdin.

Biliverdin does not give the van den Bergh reaction and it can be measured only by special spectrophotometric methods [1911]. In general, bilirubin and biliverdin levels run parallel except in hemolytic jaundice in which biliverdin is absent. In complete extrahepatic cholestasis the biliverdin level is especially high, and the skin is greenish. Improvement of the nutritional status seems to reduce the serum-biliverdin level.

BILIRUBIN AND UROBILINOGEN IN URINE, FECES, AND BILE

The qualitative and quantitative determinations of bilirubin and of the sum of biliary pigment

derivatives called urobilinogen are relatively simple tests widely used in routine laboratory and screening procedures.

Bilirubin in Urine

Physiologic Basis. Bilirubin appears in the urine when the prompt-reacting serum bilirubin is elevated, as measured by the methods in clinical use. The complex relations between serum and urinary bilirubin have been previously discussed (see Bilirubin, Chap. 11).

Methods. The qualitative demonstration of bilirubin is very useful, while the quantitative methods, using methylene blue [1066], diazo reaction [1204, 1226], or oxidation to biliverdin [395, 3321], are still restricted to academic studies. If possible the morning urine or 2-hour afternoon specimens should be examined to avoid errors owing to excessive dilution. A yellow color of the foam after shaking is suggestive, but not diagnostic, of bilirubinuria.

OXIDIZING REAGENTS. The demonstration of bilirubin with the help of oxidizing agents such as nitric acid (the Gmelin-Rosenbach test) or tincture of iodine (the Smith test) is more specific. In the latter test, a layer of tincture of iodine is put over the urine in a test tube. A green ring at the interphase indicates biliverdin as a result of oxidation of bilirubin. If the oxidized urine is filtered, the filter paper is stained green.

METHYLENE BLUE. Addition of methylene blue to urine has been recommended as a means of demonstrating bilirubin [2395]. A green discoloration results from mixture of the yellow bilirubin color with methylene blue. This is not a chemical reaction and is therefore of questionable specificity, because other yellow pigments, such as Atabrine or riboflavin, also cause a green color [3229]. The results are similar to those of the "foam test."

ABSORPTION TESTS. The absorption of the urinary bilirubin on various precipitates and its demonstration by diazo reagent [310] or by oxidation to the blue bilicyanin [2407] are of greater specificity. Commercially prepared tablets containing diazo reagent are available [1177, 3127]. The Harrison test, using barium chloride and Fouchet's reagent as an oxidizing agent, has been standardized with filter paper strips [3507] or plaster of paris tablets [1071] as a semiquantitative method [3507]. The absorption methods appear preferable, since they avoid errors caused by drugs such as chlorpromazine excreted in the urine.

Technique for Harrison Test Using Tablets. **PREPARATION OF PLASTER OF PARIS TABLETS.** Commercial plaster of paris, about 350 gm, is mixed with 250 ml saturated solution of barium chloride. The mixture is stirred and kneaded until it is homogeneous. It is then poured before the plaster sets into a suitable rubber mold, such as the Rubber Maid Door Mat manufactured by the Wooster Rubber Company, Wooster, Ohio, containing about 250 indentations, each of which is 5 mm deep and 18 mm in diameter. The surfaces are then evened off, and the plaster is allowed to set. After the tablets are firm the mat is inverted and the tablets drop out. They may be further dried in an oven.

FOUCHET'S REAGENT. Dissolve 25 gm trichloroacetic acid in 200 ml distilled water and add 10.0 ml of a 10 per cent ferric chloride solution.

PROCEDURE. Using an eye dropper, slowly drop 10 drops of urine on the surface of a tablet. Bilirubin if present is absorbed on its surface, discoloring it slightly yellow, while the rest of the urine filters through. Then drop two to three drops of the Fouchet's reagent on the discolored spot on the tablet. A positive test is denoted by a blue-green color which varies in intensity with the amount of bilirubin present. The results must be read immediately after the addition of Fouchet's reagent and are graded from 0 to 4+. Occasionally a gray or lavender color develops in concentrated urine. This is caused not by bilirubin but by other pigments derived from urobilinogen.

Results. With the methods routinely used, no bilirubin is found normally, while with sensitive methods traces are always found [2407]. In dogs, bilirubin is normally present in the urine. In jaundice resulting from cholestasis or from hepatic-cell degeneration, bilirubin is found in the urine, while in hemolytic jaundice or "acholuric" jaundice it is absent, even with bilirubin levels as high as 9.0 mg per 100 ml serum. More bilirubin is present in the urine with equal serum-bilirubin levels in cholestasis than in hepatic-cell damage [2775], suggesting a greater clearance of bilirubin in cholestasis or renal impairment in hepatic-cell degeneration. In renal failure bilirubin may be absent from the urine even in severe jaundice. In the initial stages of both extrahepatic biliary obstruction and hepatitis, bilirubin may appear in the urine when the serum bilirubin is hardly elevated [1146, 2409, 3502] (Table 38). In the defervescent stage or after relief of obstruction, bilirubinuria may disappear while the serum-bilirubin level is still elevated.

Evaluation. The qualitative methods for urinary bilirubin suffice for most practical applications,

such as (1) screening for initial stages of viral or toxic hepatitis, of importance in military or industrial medicine; (2) recognition of early extrahe-

Table 38 Diagnostic Significance of Bilirubinuria

- I. Hepatic diseases associated with bilirubinuria
 - A. Cholestasis
 - B. Hepatitis
 - C. Cirrhosis
 - D. Fatty liver with jaundice
- II. Extrahepatic conditions associated with bilirubinuria
 - A. Congestive heart failure
 - B. Pulmonary infarcts
- III. Hepatic diseases in which bilirubinuria may be the only laboratory clue to diagnosis
 - A. Preicteric and anicteric hepatitis
 - B. Fatty liver
 - C. Cirrhosis without jaundice
 - D. Early cholestasis
- IV. Diffuse hepatic diseases in which bilirubinuria is not found frequently
 - A. Late, even icteric, stages of hepatitis
 - B. Cirrhosis even with mild jaundice
 - C. Any hepatic disease with renal failure

Note: Bilirubinuria is also absent in uncomplicated hemolytic jaundice and in familial nonhemolytic jaundice.

patic biliary obstruction before jaundice becomes manifest; (3) following the course of hepatic diseases, replacing serum-bilirubin determinations; (4) differentiation of pure hemolytic jaundice from other types of jaundice, replacing the van den Bergh reaction in serum; (5) the recognition of renal impairment in jaundice.

Bilirubin in Bile and Feces

Estimation of bilirubin concentration in bile or duodenal juice has been made chiefly with the use of the icterus index or other methods which are not necessarily reliable. Recently, more accurate procedures have become available [3638]; these are also useful in animals [482]. For qualitative analyses, the methods used to demonstrate bilirubin in the urine are applicable. Bilirubin appears in the feces in diarrhea, in newborn infants fed breast milk, or because of suppression of bacterial action. For its demonstration, the blue color after the addition of Fouchet's reagent to a 1:20 stool dilution, and the green biliverdin color with Schmidt's sublimate test or Gmelin's nitric acid test are used.

Urobilinogen in Urine

Physiologic Basis. Urobilinogen in the urine designates a group of colorless pigment precursors,

especially mesobilirubinogen and stercobilinogen. Both are probably formed from bilirubin in the intestine by bacteria and partially absorbed into the blood stream, from which much is taken up by the liver and excreted into the intestine. Normally little is excreted in the urine. Urobilinogen can be considered a single entity for practical clinical purposes, since each component gives the same reactions with most methods used in simple urinalysis. With increased blood destruction, increased amounts of bilirubin are formed and excreted into the intestine, where an increased amount of urobilinogen is formed and absorbed. If the capacity of the liver to take it up is exceeded, the surplus is excreted in the urine. In hepatic-cell degeneration the urinary excretion is increased because the enterohepatic circulation is interrupted. Increased urinary urobilinogen, therefore, indicates either hepatic-cell degeneration or hemolysis.

In cholestasis, less bilirubin than normal or none at all enters the intestine, and less urobilinogen than normal or none at all is formed, absorbed, and excreted in the urine. If renal failure can be excluded, reduced or absent urinary urobilinogen indicates cholestasis. Some urobilinogen is formed even with complete biliary obstruction by bacterial action upon bilirubin in desquamated epithelial cells and in intestinal secretions, or by transformation of bilirubin in the biliary passages in the presence of bacterial cholangitis usually associated with stasis.

Since hepatic-cell degeneration, cholestasis, and increased blood destruction often occur simultaneously, the urobilinogen excretion may be difficult to interpret. Urobilinogen is transformed by bacteria to orange-yellow urobilin either in the bladder in cystitis or more commonly upon standing in the open. If urobilin is present in large amounts, the urine is dark but the foam is not yellow.

Methods. For qualitative analysis, either urobilinogen is measured with Ehrlich's aldehyde reagent, which produces a pink color, or urobilin, which gives a green fluorescence when mixed with zinc acetate, is determined. Ehrlich's aldehyde reaction is not specific because the red color can also be produced by indole, skatole, porphobilinogen, and Pyridium compounds. The urobilinogen tests are reliable only when performed on freshly voided urine before transformation to urobilin occurs, whereas for the urobilin determination, urobilinogen must be oxidized to urobilin by adding tincture of iodine. The results of both methods

depend upon the dilution of the urine. The examination of morning specimens or specimens collected at fixed times avoids errors in interpretation, which readily occur with random specimens.

For most diagnostic procedures the qualitative test suffices. The urobilinogen test appears preferable, because absence of pigment is more easily recognized by lack of a color reaction than by lack of fluorescence. The urobilin test can be used in circumstances when freshly voided urine is not available or for the exclusion of nonurobilinogen colors, which develop with Ehrlich's aldehyde reagent.

Qualitative Tests. UROBILINOGEN. The simplest procedure is the addition of five to eight drops of Ehrlich's aldehyde reagent (5.0 gm para-dimethylaminobenzaldehyde dissolved in 50 ml each of concentrated hydrochloric acid and distilled water) to freshly voided urine in a test tube and inspection within 1 minute. A barely recognizable pink color, more readily seen by looking down into the test tube, indicates a normal urobilinogen content. The color becomes more prominent upon heating. Increased urobilinogen is recognized by a cherry-red color, which becomes dark red upon heating. A pink color appearing only on heating indicates decreased amounts. Absence of any pink color even upon heating signifies absence of urobilinogen. The pink urobilinogen color can be concentrated by adding to the test tube chloroform, which does not extract porphobilinogen [3514]. A green color which develops immediately in jaundiced patients results from oxidation of bilirubin to biliverdin. This is accomplished by the hydrochloric acid of the reagent in the presence of nitrites formed by bacteria in the urine in infection or in standing specimens [1638]. The reaction is then best performed in urine after precipitation of bilirubin by adding 1.0 ml 10 per cent calcium chloride solution to 4.0 ml urine, and filtering. The specificity of the red color for urobilinogen can also be substantiated by adding 4.0 ml saturated sodium acetate solution to one urine aliquot before adding the Ehrlich's reagent and to another 15 seconds after adding the Ehrlich's reagent [1719]. The urobilinogen color develops only in the specimen to which acetate is added after the Ehrlich's aldehyde reagent. The presence of Pyridium is recognized by the red color that develops on the addition of hydrochloric acid alone.

UROBILIN. To about 5 ml urine a few drops of Lugol's solution are added. Then 5.0 ml saturated alcoholic solution of zinc acetate (approximately

10 gm in 100 ml ethyl alcohol) is added. The solution is filtered, and the filtrate is viewed in a test tube in bright light against a dark background. A greenish fluorescence indicates urobilin. In icteric urine bilirubin interferes with the fluorescence and must be removed by adding calcium chloride, as described above. Many substances which give a color reaction, such as Pyridium, do not give the urobilin reaction. Fluorescent compounds, such as riboflavin, give the reaction but can be identified by specific procedures [2407].

Semiquantitative Methods. Semiquantitative procedures for the determination of urinary urobilinogen are based on the red color that develops with Ehrlich's aldehyde reagent. The simple dilution of the urine originally recommended is now modified by diluting the reaction mixture. The most widely used of these semiquantitative methods is based on the determination of the urine in a 2-hour sample collected between 2 and 4 P.M., a time at which the urobilinogen excretion is most constant [3429, 3515]. Diluted Ehrlich's aldehyde reagent and, after 15 seconds, saturated sodium acetate solution are added to an aliquot [1719]. Urine to which sodium acetate is added before the Ehrlich's aldehyde reagent serves as a blank. The readings are made either with a colorimeter calibrated with pontacyl red [3515] or with a comparator using dilutions of pontacyl red or phenosulfonphthalein [395]. Since nonurobilinogen colors are also measured, the values are expressed in units, the normal range being between 0.2 and 0.8 units in the 2-hour specimen [3515]. However, false negative results appear frequently [3429, 3507]. Refrigeration does not interfere with the test [1812]. Since it is not superior to the simple qualitative test, for exact information a 24-hour quantitative determination is recommended.

Quantitative Methods. Watson's method [2964, 3502] is based on the Ehrlich's reaction in an acidified petroleum ether extract of a 24-hour urine specimen, in which the urobilin is oxidized to urobilinogen, and is preferable to simpler methods, using individual specimens and gold chloride standards [3142, 3179, 3504]. Stercobilinogen and mesobilirubinogen were originally used for calibration, but these have been replaced by pontacyl red. Bright sunlight interferes with the determination [3427].

Technique for Quantitative Method. REAGENTS

1. Sodium carbonate, anhydrous
2. Petroleum ether

3. Ferrous sulfate solution, 20 per cent, freshly prepared
4. Sodium hydroxide solution, 10 per cent
5. Ehrlich's reagent, 1.4 gm para-dimethylamino-benzaldehyde dissolved in 300 ml concentrated hydrochloric acid and 200 ml distilled water
6. Saturated sodium acetate solution
7. Glacial acetic acid

COLLECTION OF SPECIMENS. Collect the specimen in a dark bottle containing approximately 5.0 gm sodium carbonate under a layer of petroleum ether to protect it from the air.

PROCEDURE. To 50 ml of the sample in a 125-ml Erlenmeyer flask, add 25 ml ferrous sulfate solution and 25 ml sodium hydroxide solution. Mix this well and place in the dark for 1 hour, then filter. The amount to be used is determined by the simple qualitative test. To 2.0 ml of the filtrate add an equal amount of Ehrlich's aldehyde reagent. Then add 4 to 6 ml sodium acetate solution. If the color is dark red, 1 to 2 ml filtrate will suffice; if pale red, 5 to 10 ml; if pink, 15 to 25 ml; and if colorless, 50 ml. Place the amount necessary in a separatory funnel; if it is small, dilute it with distilled water to at least 25 ml. Cover it with about 50 ml petroleum ether and acidify with 5.0 ml glacial acetic acid. Shake vigorously and allow to settle. Decant the ether from the top of the funnel after letting the aqueous fraction through the lower outlet. Extract the aqueous fraction twice more with the same amount of ether and then place all the ether used in a clean separatory funnel and wash with a small amount of distilled water, which is then discarded. To the ether, add 2.0 ml Ehrlich's aldehyde reagent and shake vigorously. Exactly 15 seconds after adding the Ehrlich's reagent, add 6.0 ml of the sodium acetate solution and again shake the mixture well. A fine reddish-brown precipitate at the interphase at this time is caused by indole or skatole. Place the colored aqueous fraction after complete separation in a 100-ml graduated cylinder. Repeat the extraction of the ether with Ehrlich's reagent and sodium acetate until the color in the final aqueous fraction is faint. Then make up the volume in the graduated cylinder to a convenient volume with distilled water. Make readings on 10 ml of the final solution at a wavelength of 565 with a blank consisting of 9.0 ml sodium acetate solution and 3.0 ml Ehrlich's reagent. The standard curve is made by using known concentrations of urobilinogen or sterco-bilinogen [400] or by using a pontacyl dye standard [3429, 3515].

CALCULATION.

$$\frac{100}{50} \times \frac{\text{vol. final solution}}{\text{vol. filtrate used}} \times \text{concentration} = \text{mg per 100 ml urine}$$

Multiply this result by the volume of urine in 24 hours for the total excretion per day.

Results. The normal values with quantitative methods vary between 0.2 and 3.0 mg per day, usually averaging between 0.5 and 1.5 mg [3179, 3502], and are seldom less than 0.2 mg. Several determinations on consecutive days are advantageous. The excretion varies throughout the day in different individuals [2555].

HEPATITIS AND CIRRHOSIS. In acute hepatitis the urinary urobilinogen excretion is usually significantly elevated [491, 2010, 2897, 3179, 3502] (Table 39). In many instances, after an initial rise

Table 39 Diagnostic Significance of Urinary Urobilinogen Excretion

- I. Hepatic diseases associated with increased urinary urobilinogen excretion
 - A. Hepatitis
 - B. Cirrhosis
 - C. Intermittent and incomplete extrahepatic cholestasis
 - D. Carcinoma
 - E. Fatty liver
- II. Extrahepatic conditions associated with increased urinary urobilinogen excretion
 - A. Hemolytic conditions including pernicious anemia, transfusion reactions
 - B. Congestive heart failure
 - C. Fevers, infections
- III. Hepatic diseases in which increased urinary urobilinogen excretion may be the only laboratory clue to diagnosis
 - A. Anicteric hepatitis
 - B. Cirrhosis without jaundice
 - C. Toxic hepatitis
- IV. Diffuse hepatic diseases in which urinary urobilinogen may be low or absent
 - A. Extrahepatic and intrahepatic cholestasis
 - B. Hepatic diseases associated with anemia, especially cirrhosis
 - C. Hepatic diseases complicated by renal failure
 - D. Some stages of hepatitis
 - E. Fatty liver

Note: Antimicrobial therapy may alter intestinal flora so that no urobilinogen is formed.

above normal the urobilinogen drops below normal during a stage of severe cholestasis. In the recovery period the urobilinogen rises again to abnormal levels. Curves drawn from estimations of urinary bilirubin and urobilinogen cross each other twice [3185, 3502] (Fig. 78, lower). The first crossing occurs when bilirubin rises and urobilinogen drops in the early stages of the disease. In the defervescent stage the urobilinogen rises and the bilirubin drops (see Laboratory Findings, Chap. 43). Serial determinations are helpful in the recovery period, as well as in chronic hepatitis, because the urobilinogen excretion may remain abnormal

longer than the results of many other tests, indicating persistent hepatic injury [2010, 2897]. Also in the prodromal stage or in the acute anicteric phase, the increased urobilinogen excretion is of diagnostic value [491]. In cirrhosis with jaundice urinary urobilinogen is usually increased [3502], but with complicating cholestasis it may be reduced or absent [2555, 3179, 3183]. In cirrhosis without jaundice, urobilinogen excretion is usually high. Low values are occasionally recorded, particularly if anemia is present. In intrahepatic cholestasis, or "cholangiolitis," urobilinogen excretion may be increased, normal, or decreased.

BILIARY OBSTRUCTION. In extrahepatic biliary obstruction from carcinoma, urobilinogen is absent [3179, 3502] except in the presence of complicating cholangitis or if the obstruction is relieved, either by operation or sloughing of the tumor. In calculous obstruction the excretion varies, depending upon the location of the stone or the presence of infection or biliary cirrhosis [3502]. When a stone completely obstructs the common duct, excretion stops. If the obstruction is incomplete, urobilinogen is increased, because the hepatic uptake of urobilinogen from the blood stream is interrupted. The excretion is especially increased during episodes of intermittent obstruction producing typical spiking curves (Fig. 78, upper). Therefore, results of single determinations are often misleading. The urobilinogen excretion is increased in strictures, particularly when associated with cholangitis.

OTHER CONDITIONS. In uncomplicated hemolysis, the urobilinogen excretion in the urine is not necessarily elevated. It is increased in the presence of liver damage, hemolytic crisis, and many other complications [3179, 3502]. In chronic passive congestion and with extensive carcinoma metastases, urobilinogen excretion is increased because of disturbed hepatic uptake [990]. Impaired hepatic function as a result of stress is said to account for urobilinogenuria in acute myocardial infarction [960]. In infectious mononucleosis and in many other infections, such as pneumonia, urinary urobilinogen is elevated [1661]. This is probably a sensitive indicator of hepatic-cell degeneration.

If advanced renal disease is present, urinary urobilinogen is reduced or absent in conditions which ordinarily increase its excretion. After antibiotic therapy the urobilinogen excretion may be low or absent for a considerable period because of the suppression of the colonic flora [2896]. Dextrorotatory urobilinogen appears temporarily

after discontinuation of the antibiotic administration (see Nomenclature and Chemistry of Bile Pigments, Chap. 11). Urobilinogen is absent in newborn infants, because no bacteria have entered the colon. In diarrhea, urobilinogen may be absent, because bilirubin has not remained in the intestine long enough to be transformed and absorbed.

Evaluation. Urobilinogen determinations are of value in many circumstances if complicating factors are taken into account. In jaundiced patients serial determinations, for which the qualitative or semiquantitative methods suffice, are recommended. The quantitative determination should be used for clarification of discrepancies and for scientific purposes. Increased urobilinogen excretion is indicative of hepatic-cell degeneration if hemolysis can be excluded, and it assists in screening for hepatitis in otherwise healthy people. Urobilinogenuria reflects the degree of hepatic congestion in cardiac decompensation. Secondary hepatic involvement in intoxications or infections in the absence of jaundice increases urobilinogen excretion. In the presence of jaundice values below 0.2 mg per day indicate extrahepatic biliary obstruction, with the exception of instances of intrahepatic cholestasis, or "cholangiolitis." Serial determinations assist in the differentiation of calculous obstruction, with fluctuating excretion, from malignant obstruction, with no excretion. Hepatitis has a biphasic curve.

Urobilinogen-tolerance Test

A urobilinogen-tolerance test is considered too complicated for general use [3638]. The increase of urinary urobilinogen after the intravenous injection of stercobilin crystals prepared from human feces indicates the capacity of the liver to take up urobilinogen [3502]. None appears in the urine under normal circumstances, in contrast to the condition present in liver damage. The test is not used when more than 5.0 mg urobilinogen is excreted in the urine in 24 hours. Standardized solutions are difficult to prepare.

Biliary Urobilinogen

Compounds of the urobilinogen group have been demonstrated in bile or duodenal juice [943, 3638], but their chemical nature is still unsettled. Some are dextrorotatory, particularly in infection, and demonstration of urobilinogen in bile has been suggested as a test for infection in the biliary tract [943] or in the gallbladder. Since liver damage

cannot be excluded as a cause of "urobilinocholie," the test is not recommended. Comparison of urobilin and bilirubin in bile has also been suggested as a test for cholecystitis, since impaired urobilin absorption by the damaged gallbladder supposedly lowers the normally high bilirubin/urobilin ratio [2842].

Fecal Urobilinogen

Physiologic Basis. Compounds of the urobilinogen group similar to those in urine are found in the stools, with stercobilinogen usually predominating. In contrast to the situation in urine, most of the compounds are in the colored oxidized form, as the pigments largely responsible for the brown color of the normal feces. Biliary obstruction decreases fecal urobilinogen, while hemolysis increases it. Hepatic-cell damage, important in urinary urobilinogen excretion, does not influence it.

Methods. Methods similar to those used in urine are applied to determine fecal urobilinogen. Qualitative tests are performed to demonstrate the presence or absence of biliary secretion, since bilirubin is usually transformed to urobilinogen or urobilin. Urobilinogen can be determined by addition of Ehrlich's aldehyde reagent to an acid petroleum ether extract of the stool which does not contain indole and skatole. This method fails to detect the bulk of the pigment, which is oxidized urobilin. The zinc acetate method of Schlesinger can be used on a similar extract but requires inspection for fluorescence, which makes it unreliable for small amounts. Color reactions of urobilin are preferable, especially the Schmidt test, demonstrating both urobilin and bilirubin.

SCHMIDT TEST. Rub a pea-sized piece of feces into a petri dish and mix with a 5 per cent aqueous solution of mercuric chloride. Cover the dish and observe after several hours, preferably 1 day. Urobilin produces a pink color, while bilirubin gives a green biliverdin color. No color after 24 hours indicates absence of bile pigments. In the presence of much fat, preceding ether extraction is required.

Quantitative methods are applied to confirm qualitative results, to demonstrate increased urobilinogen formation, and to compare with urinary urobilinogen excretion. Therefore, semiquantitative methods, such as serial dilution of the Ehrlich's aldehyde color reaction, simple comparison with gold chloride standards [3142], or estimation of the color without petroleum ether extraction [3515], are not recommended. The more reliable

method [2964], which uses the same procedure given for the quantitative determination in the urine [2116, 3179, 3582], should be used. It is based on reduction with ferrous sulfate and subsequent petroleum ether extraction. For comparison with urine urobilinogen, examination of individual stool specimens of more than 250 gm solid feces is as informative as the collection of stools for several days [3179].

Results. In normal adults the fecal excretion of urobilinogen varies widely from day to day. It is usually between 40 and 300 mg per day [3502], averaging between 100 and 200 mg. In children the values are lower. On high-fat diets, fecal urobilinogen is increased, possibly because of increased hemolysis from fatty acids and soaps [1663]. In hepatitis and cirrhosis, as well as in incomplete obstruction resulting from stones, the values vary between 10 and 300 mg per day, although the excretion is usually reduced. In ball-valve obstruction, fluctuating values are found. In malignant obstruction, which is usually complete, the values vary between 0 and 5.0 mg per day [3179]. After antibiotic therapy less than 5.0 mg per day may be excreted [2896]. In hemolytic anemias, the values vary between 300 and 1,800 mg per day. Similar values are seen in primary hepatic disorders complicated by blood destruction. During a hemolytic crisis the excretion may rise to 2,500 mg per day.

Serial determinations of fecal urobilinogen permit an accurate evaluation of blood destruction, especially if compared with the circulating hemoglobin, the hemolytic index [2288]. The relation between daily urinary urobilinogen excretion and the daily fecal urobilinogen excretion [3502] or the fecal urobilinogen concentration [3179] can be expressed as a ratio, which is helpful in the separation and appreciation of liver damage and hemolysis. With increased urinary urobilinogen from hemolysis, the ratio between fecal and urinary urobilinogen is higher than normal, since much of the excess urobilinogen formed and absorbed is taken up by the liver. In contrast, urobilinogenuria from hepatocellular degeneration is characterized by a low fecal/urinary ratio, since the damaged liver is unable to take up and re-excrete the normal amount of urobilinogen absorbed. In hepatocellular damage, even if the fecal urobilinogen is reduced by associated cholestasis, the urinary urobilinogen remains relatively high, and the fecal/urinary ratio is therefore low. This does not permit differentiation from incomplete

biliary obstruction, in which the fecal urobilinogen is reduced and the fecal/urinary ratio is also low, owing to associated liver damage.

Evaluation. The qualitative demonstration of fecal urobilinogen compounds is a simple test for biliary secretion into the intestine, although inspection of the feces and testing the urine for urobilinogen can usually substitute for this procedure. Quantitative determinations give evidence of increased blood destruction if used in comparison with hemoglobin levels. They also assist in the separation of hemolytic from hepatic causes of increase in urinary urobilinogen. For this, as well as for scientific studies, an elaborate method is required.

PORPHYRINS

Physiologic Basis. Porphyrins are key intermediates in the formation of hemoglobin and respiratory enzymes. Coproporphyrin, one of these intermediates, exists in two isomeric forms in the body, each of which has a different biologic significance. Coproporphyrin I is a by-product of hemoglobin synthesis and is discarded in the urine and bile. Coproporphyrin III is one of the stages in the formation of hemoglobin. Surplus amounts of this isomer are also excreted normally in urine and bile [3503]. In liver diseases the biliary excretion of coproporphyrin I is impaired, because of either hepatic-cell damage or regurgitation, and increased amounts appear in the urine. Coproporphyrin III is formed in excessive amounts in toxic disorders; for instance, in lead poisoning [1522, 2932] or alcoholism [3268]. Total urinary coproporphyrin excretion, as well as partition of the isomers, may be of value for the diagnosis and prognosis of liver disease [804, 3508].

Methods. Most of the methods used for determination of urinary coproporphyrins involve extraction of relatively large amounts of urine with ether, followed by measurement of fluorescence [2963]. These methods have been criticized because they are complex and subject to many tech-

nical difficulties [2966]. In addition, results obtained are uniformly low, because a colorless precursor of coproporphyrin, coproporphyrinogen, which may account for the bulk of the urinary porphyrin, is not measured [3505]. Recently a new method was introduced which utilizes only 5.0 ml aliquots of 24-hour alkalized urine specimens extracted with ethyl acetate and buffered acetic acid [2966]. The coproporphyrinogen is converted to coproporphyrin by iodine solutions, and readings are made on a sensitive fluorophotometer. Reproducible results are obtained with this relatively simple method. For the isomeric partition the earlier, more complex method is required.

Results. Normally 60 to 280 μ g of total coproporphyrin, as determined by the newer method [2966], is excreted daily in the urine [3503]. This amount is considerably increased in some anemias and in heavy-metal poisoning. In viral hepatitis, infectious mononucleosis, and obstructive jaundice, the total urinary coproporphyrin increases to 800 to 1,000 mg per day. The greatest part of the increase is coproporphyrin I [3508]. In viral hepatitis the elevation may persist longer than other laboratory or clinical findings [3267]. In cirrhosis, increase of total coproporphyrin is also found. It does not parallel the clinical picture or the bilirubinuria, but it appears to be a sensitive index of hepatic damage. In nonfatty, posthepatic cirrhosis or hemochromatosis, isomer I predominates, whereas in alcoholic fatty cirrhosis, isomer III predominates [3517]. Alcohol abuse alone increases the isomer I excretion [3268], which may persist for days after the alcohol intake. The urinary/fecal coproporphyrin ratio is elevated in liver damage [2039, 2431], suggesting a behavior similar to that of urobilinogen.

Evaluation. Urinary coproporphyrin determinations may be helpful as an index of chronicity of hepatic injury but not in the differential diagnosis of jaundice. Isomer partition permits the differentiation of fatty nutritional cirrhosis from other types of cirrhosis, although the methods are still too elaborate to permit their routine use.

TESTS BASED ON BILE ACID METABOLISM

The bile acids are formed by the hepatic cells and enter the blood and bile. Excessive amounts in the blood are excreted in the urine. The biliary bile acids are partly reabsorbed in the intestine and reexcreted by the liver in the enterohepatic circulation. In hepatectomized dogs, bile acids disappear from the blood and urine and injected bile acids are completely recovered from the urine. In hepatic-cell degeneration, bile acid formation is reduced. The reduced serum level may be compensated for by impaired biliary excretion, as well as by faulty hepatic uptake of reabsorbed bile acids, leading to increased blood and urine levels. In extrahepatic cholestasis, regurgitation leads to elevation in level of serum bile acids and to their excretion in the urine. In hemolytic jaundice, bile acid levels in blood and urine are normal.

Serum Bile Acids

Methods. Several methods have been described for measurement of cholic acid, the most common being the Pettenkofer reaction or a modification of it; the development of a violet color after the addition of sulfuric acid and fructose or furfuraldehyde [1598, 1664]. Of the bile acids, only cholic acid gives this reaction, not conjugated acids or desoxycholic acid, although it is also given by some nonbiliary steroids. The determination of total bile acids is based on the hemolytic properties of bile salt extracts from blood [1996]. Partition of cholic and noncholic bile acids has been attempted [1996].

Results. Great variations have been recorded in the normal values; with Josephson's method [1664] the levels vary between 0.2 and 4.0 mg

per 100 ml of serum, with a mean of 1.5 mg per 100 ml for cholic acid [1664, 3045]. In acute hepatitis the values are slightly higher, rising to 4.4 mg per 100 ml, with a mean of 2.76 [1664, 3045, 3378]. In cirrhosis variable results have been reported [1664], the mean being 1.96 mg per 100 ml in active forms, and 1.4 mg per 100 ml in latent cirrhosis [3045]. In extrahepatic cholestasis, especially with complete obstruction, the highest values are reported, the mean being 4.0 mg per 100 ml [3045]. In prolonged obstruction, the levels drop [1598]. The overlapping between obstructive jaundice and hepatitis is too great to permit diagnostic use of the test.

Bile Acids in Urine

Several qualitative methods for the demonstration of bile acids in urine have been described.

1. The Pettenkofer reaction. To about 5 ml urine, five drops of 5 per cent sucrose solution is added. After mixing, 3.0 ml concentrated sulfuric acid is carefully underlaid. A red ring at the interphase or a gradually developing red color after mixing and cooling indicates a positive reaction.

2. Hays test. Bile acids reduce surface tension of liquids, and if flowers of sulfur are sprinkled on the top of the urine, the particles sink if bile acids are present. If the urine contains more than 0.5 mg per 100 ml, they sink rapidly, but if less than 0.25 mg is present, they do not sink at all, even after agitation [1996]. Other surface-active substances, especially some drugs, cause the same phenomenon.

Quantitative methods include stalagmometry [2354] and adaptations of the Pettenkofer reaction [1996]. The demonstration of bile salts in the urine is of interest in showing the dissociation of bilirubin and bile acid excretion, but it offers little

assistance in the recognition of impaired liver function [2354].

Bile Acids in Bile and Feces

Bile acids in bile have been demonstrated by several techniques, primarily for academic reasons. Only occasionally have these techniques been used after duodenal drainage or on biliary fistula bile for clinical purposes [1996]. In general, gallbladder bile contains a greater concentration of bile acids than liver bile because of concentration within the gallbladder. The difference is not so great as with bilirubin, because the gallbladder wall absorbs some bile acids [1664]. In liver damage the bile acid concentration is reduced [2354] and the distribution of bile acids is reportedly altered [817]. In patients with biliary fistulas, bile salts demonstrable by the Pettenkofer reaction may be absent for a considerable period of time after release of the obstruction. This results from liver damage because of the prolonged obstruction and may have prognostic significance [1664, 2721].

Bile acids in feces, determined by the Pettenkofer reaction, suggest altered intestinal absorption rather than hepatic dysfunction.

Cholate-tolerance Test

Intravenously injected sodium cholate remains in the blood longer than normal in both hepatocellular degeneration and cholestasis, a greater amount remaining in the former [1664]. In liver disease without jaundice this increase in blood cholates is very slight.

Evaluation of Tests Concerning Bile and Metabolism. In view of the opposing trends influencing the serum-bile acid level, the measurement of bile acids in the blood and their variations after administration of a test dose have little significance in the recognition of liver damage or in the differential diagnosis of jaundice. Similarly, demonstration of bile acids in the urine has chiefly academic interest. Determination of bile acids in fistula bile is a practical method of evaluating the degree of injury and the rate of recovery after biliary surgery.

TESTS CONCERNING VITAMIN A, MINERALS, AND ELECTROLYTES

The relation of the liver to the metabolism of vitamin A, minerals, and electrolytes is reflected in hepatic tests, the practical application of which

is limited because of the influence of other organs upon the metabolism of these substances.

Plasma Vitamin A

Physiologic Basis. Vitamin A is brought from the intestine to the liver in the ester form and stored there as such. It is released to the blood for utilization by the tissues as vitamin A alcohol. In hepatic injury the absorption of vitamin A and carotene from the intestine is impaired, and the liver depots are gradually reduced. Eventually this results in decreased release of vitamin A from the liver and reduction of the blood levels. The disturbance of release of vitamin A from the liver, possibly because of impaired ester hydrolysis, is of even greater importance in liver disease. This endogenous hypovitaminemia rapidly develops in acute hepatic injury, and during recovery temporary hypervitaminemia may develop. The carotenoid level depends mainly upon the nutritional intake, rather than upon release from the liver.

Methods. Vitamin A dissolved in chloroform gives a rapidly fading blue color with antimony trichloride (the Carr-Price reaction). This is performed on chloroform solutions of the residues of petroleum ether extracts of plasma. The color is read either colorimetrically [1751] or in a visual comparator [1828] with copper sulfate standards [1662]. Carotenoids, which are not necessarily identical with biologically active beta carotene, are determined by measuring the color of the petroleum ether extract directly [1751]. Since the carotenoid pigments also exhibit the Carr-Price reaction, although more slowly, some investigators make a correction in this reaction for carotenes. Because of the uncertainty of the effect, the deduction is often entirely omitted [2641]. Although the method gives reliable results with proper calibration, a micromethod based on specific absorption in the ultraviolet range before and after destruction of the vitamin by the light is superior [255]. The vitamin A concentration has been expressed either in units or more recently in micrograms; 1 μ g equals 3.3 international units.

Results. During fasting the plasma normally contains between 30 and 100 μ g vitamin A per 100 ml, with a mean of about 50 μ g [24, 1751, 2641]. The plasma-vitamin A level remains fairly constant in the same person [2641]. It is somewhat reduced in pneumonia or other infections [2641], in severely ill patients, and in patients with established vitamin A deficiencies or with carcinoma of the stomach [6]. In mild hepatitis the plasma-vita-

min A level is slightly reduced, but in severe hepatitis it is greatly lowered [24, 2641, 3580]. In cirrhosis, especially if associated with jaundice, the values are very low [1341, 2641]. In uncomplicated or early obstructive jaundice, the reduction is slight [24, 2641], but in infected or protracted obstruction very low levels are found [2641]. In the recovery period from acute or chronic hepatic injury, hypervitaminemia is often noted [3181]. This results in large variations of the level in cirrhosis. The plasma-vitamin A level after intake of large doses of vitamin A rises considerably less in patients with liver disease and biliary obstruction than in normal persons [24, 2646, 2696].

Plasma-carotenoid Level

The plasma contains normally between 40 and 100 μg carotenoids per 100 ml, with a mean of 80 μg [2641]. The carotenoid levels and the response to intake of carotenoids are not clearly related to hepatic disorders, although in cirrhosis low values have been reported [21, 24]. The vitamin A/carotenoid ratio is reduced in severe hepatic disorders, possibly because of impaired hepatic uptake of carotene [2641].

Serum Electrolytes and Minerals

Determinations of serum sodium, potassium, and chloride are important in hepatic disorders from a therapeutic viewpoint, but abnormal results do not indicate hepatic dysfunction. The same holds true for other electrolytes or minerals, with the exception of serum iron, determination of which has recently been recommended as a hepatic test.

Serum Iron. The liver is the main site of iron storage in the body, and in acute liver damage stored iron is released to the blood, increasing the serum-iron level.

Normally serum contains between 60 and 200 μg iron per 100 ml [581, 857, 1363, 2576]. Evening values are almost half of fasting morning levels, regardless of the initial iron concentration [1363]. Men have slightly higher values than women [857]. Hemolyzed specimens should not be used. In acute hepatitis the iron level is greatly increased, especially in the first 2 weeks, in contrast to what occurs in cirrhosis and cholestasis, although some overlapping is seen [581, 857, 2239, 2576]. The serum-iron elevation does not parallel the degree of hepatic injury or of bilirubinemia [1280, 2239], but it has been claimed to

parallel the clinical picture [581, 2239]. In hepatitis from infectious mononucleosis, malaria, and brucellosis, the elevation is supposedly not so great as from viral hepatitis [581]. In fatty metamorphosis and cirrhosis the level is usually normal, except in severe hepatocellular degeneration. In hemochromatosis, the serum-iron level is often increased [1552], but not necessarily so [1181]. Hepatic disorders do not alter the results of iron-tolerance tests [3401].

Water Metabolism

Physiologic Basis. Water retention reflected by reduced urinary volume and edema occurs in many hepatic disorders, especially in cirrhosis, largely because of sodium retention. Water retention is therefore caused either by reduced hepatic neutralization of hormonal antidiuretic principles or by a disturbed action of the hepatic vasculature regulating the water content of the liver and thus in turn influencing the blood volume and the fluid space.

Results. Charting intake and output demonstrates water retention in the acute stages of hepatitis or in cirrhosis, especially when associated with ascites [1654]. In the recovery period of hepatitis, compensatory diuresis is usually noted.

Following the intake of 1,000 to 1,500 ml water, the urinary excretion is less than normal in patients with liver disease [18, 2697]. Diuresis is often delayed, and the specific gravity of the urine may not drop. This response occurs in hepatitis and in prolonged obstruction and is absent in uncomplicated biliary tract disease [18]. In cirrhosis with ascites the excretion is less than in cirrhosis without ascites [2697].

The results of water-tolerance tests are influenced by such factors as edema, ascites, dehydration, and renal damage [1893].

TRANSFORMATION TESTS

The liver transforms many exogenous substances, and the response of the organism to the administration of almost any substance can thus be used as a hepatic test. Many hepatic tests based on this fact have been described. The transformation follows one of several pathways, such as destruction, oxidation, or conjugation with various substances. The conjugation process occurs mainly in the liver and is considered a detoxification; although usually it only facilitates

urinary excretion. Only the most widely used tests are described here.

Hippuric Acid Synthesis

Physiologic Basis. Among the conjugation tests, the most widely used is Quick's hippuric acid test [2677]. It is based on the conjugation of sodium benzoate with glycine to form hippuric acid, which is excreted in the urine. The conjugation takes place chiefly in the liver. The conjugating capacity of the kidney can not compensate for disturbed hepatic function. The rate of hippuric acid excretion by the kidney is considerably higher than the rate of hepatic synthesis [2677], but renal disorders reduce urinary hippuric acid excretion [1831, 2672]. Therefore a nearly normal NPN or urea clearance is desirable for proper evaluation of results [1831]. Hippuric acid excretion has been said to depend upon body size [2978] and upon the urinary volume [2199], although some investigators disagree [2916]. The liver controls both availability of glycine and the rate of conjugation. Reduced hippuric acid excretion in liver disease probably results from both decreased availability of glycine and faulty conjugation, since some patients fail to show an increase in hippuric acid formation as a result of glycine administration [2672]. Repeated doses of sodium benzoate increase the amount of hippuric acid formed each time, apparently because of a priming effect on glycine synthesis [3705].

Method. Originally 6.0 gm sodium benzoate was given orally and the urine collected for 4 hours [1289]. To eliminate the influence of intestinal absorption and to test the response of the liver in a shorter time, intravenous administration of 1.77 gm dissolved in 20 ml distilled water was recommended [2677], with urine collection for 1 hour.

In the determination, the hippuric acid is precipitated by acidification of the urine, the volume of which must sometimes be reduced by heating. The precipitation is facilitated by stirring and is supposed to be more complete if salts, such as sodium chloride or ammonium sulfate, are added in excess [2677, 3536]. The precipitate is filtered and washed. It is then dissolved in hot water and the amount of acid titrated, or the precipitate is dried and weighed. Technically incomplete precipitation is a common source of error [2360]; therefore any one of the described procedures

[2672, 2677, 3536] has to be followed very carefully. A paper chromatographic method using para-dimethylaminobenzaldehyde has been described [1117].

Results. Healthy individuals excrete more than 3.0 gm hippuric acid expressed as sodium benzoate, or 4.4 gm hippuric acid, in the oral test 4 hours after administration of the sodium benzoate. In the intravenous method normally more than 0.7 gm benzoic acid is excreted in 1 hour. The test can be used in children [2050]. Formation of hippuric acid is reduced in senility [2693, 3208], probably because of deficiency of glycine, rather than because of impaired hepatic function (Table 40). In late pregnancy results may be ab-

Table 40 Diagnostic Significance of Hippuric Acid Synthesis

- I. Hepatic diseases associated with decreased hippuric acid synthesis
 - A. Hepatitis
 - B. Cirrhosis
 - C. Protracted cholestasis
 - D. Carcinoma
- II. Extrahepatic conditions associated with decreased hippuric acid synthesis
 - A. Senility
 - B. Pregnancy near term
 - C. Malnutrition
 - D. Hyperthyroidism
 - E. Severe renal damage
 - F. Congestive heart failure
- III. Hepatic diseases in which decreased hippuric acid synthesis may be the only laboratory clue to diagnosis
 - A. Anicteric hepatitis
 - B. Cirrhosis without jaundice
 - C. Toxic hepatitis
- IV. Diffuse hepatic diseases in which normal hippuric acid synthesis frequently occurs
 - A. Early extrahepatic cholestasis
 - B. Cirrhosis
 - C. Hepatitis
 - D. Fatty liver

normal [1501], and a drop occurs on the first day of menstruation [1446]. In both the oral and intravenous tests, hippuric acid in excess of the amount of benzoic acid administered may be excreted [2677, 2815]. This has been considered evidence of a hyperstimulation of the liver [2815] but may also be the result of technical errors, excretion of nonconjugated sodium benzoate, or precipitation of sodium chloride [2360]. Patients with anxiety also show hyperexcretion of hippuric acid [2568].

LIVER DISEASE. Oral and intravenous tests uniformly yield abnormal results in conditions with liver damage [174, 1831, 2241, 2360, 2677, 2793, 2815, 2916, 3040] (Table 41). Since the liver

Table 41 Results of Hippuric Acid-tolerance Tests in Hepatobiliary Diseases

Diagnosis	No. cases	Mean, gm	Range, gm	% abnormal
Control patients.....	180	1.08	0.7-1.2	1.9
Acute hepatitis.....	198	0.60	0.3-1.1	70.7
Cirrhosis.....	347	0.58	0.1-1.1	84.5
Extrahepatic biliary obstruction.....	110	0.63	0.3-1.1	81.8
Hepatic tumor metastases.....	93	0.2-1.1	92.5
Chronic passive congestion.....	24	52.5
Xanthomatous biliary cirrhosis.....	16	0.70	0.0-1.2	25.0

Sources: Methods—Quick [2677], Weichselbaum and Probst [3536]; data—Bartels [173], Popper and Schaffner [2640], Quick [2677], Ricketts *et al.* [2761].

damage in biliary obstruction causes abnormal results much earlier than with most other tests, usually after 2 weeks, the test has no value in the differential diagnosis of medical and surgical jaundice [2793]. The results indicate the degree of liver damage fairly well [2241, 3626]. The oral test is less sensitive, but it is said to be helpful in the evaluation of operative risks [2241] and in following hepatic disease [2793]. A return of the results to normal in viral hepatitis follows the restoration of structural alterations [3040]. The sensitivity of the test to liver damage of any type is also reflected in the high percentage of abnormal results in hepatic metastases [2543] or in congestive heart failure. In hyperthyroidism abnormal values are found [173, 1343]. In animals results are variable because of species differences in conjugation and in the site of hippuric acid synthesis [2331].

Para-aminohippurate Synthesis. The determination of the serum levels of para-aminobenzoic acid and para-aminohippuric acid after intake of 3.0 gm sodium para-aminobenzoate, preferably together with glycine, is an index of hepatic function [757]. Para-aminohippurate synthesis is said

to be only slightly impaired in obstructive jaundice. The results are independent of renal function.

Evaluation. The oral or intravenous hippuric acid test is of no value in the differential diagnosis of jaundice. It may be helpful in estimating the degree of liver damage and in the follow-up of disease. It can be used in combination with Bromsulphalein and galactose in a single test [3709]. The results are no more revealing than those obtained with simpler methods.

Benzoyl Glucuronate Excretion

Physiologic Basis. Normal persons after ingestion of a test dose of benzoic acid excrete almost all of it as hippuric acid, while sodium benzoate as used in the hippuric acid test is more rapidly absorbed and may in part be excreted as benzoyl glucuronate [3111]. In liver damage the conjugation of benzoic acid with glycine after its administration is incomplete, and part of the benzoic acid appears in the urine as benzoyl glucuronate [348, 3111]. Also cinnamic acid is normally oxidized to benzoic acid and almost completely excreted as hippuric acid [3112]. In liver damage cinnamic acid is excreted partly as cinnamoyl glucuronate before oxidation and partly as benzoyl glucuronate, and little is excreted as hippuric acid [3108]; in addition the blood-glucuronate level is higher [2874].

Method. Benzoic acid and hippuric acid excretion can be compared quantitatively, or glucuronate can simply be demonstrated qualitatively [3112]. Three urine samples, collected 2, 4, and 6 hours after the intake of 5.0 gm benzoic acid or 3.0 gm cinnamic acid, are examined for glucuronate, by reduction methods or preferably with Tollen's naphthoresorcinol reaction [3108, 3112]. An easily visible blue hue indicates glucuronic acid in the latter reaction, while a dirty-green color is normal.

Results. Some glucuronate is normally present after 2 hours, whereas in liver damage it is found in all specimens. Its appearance in large amounts in the last specimen suggests severe liver damage. A positive test in the sample prior to administration of the benzoic acid indicates intake of other drugs. A carbohydrate meal or glucose infusions preceding the test facilitates hippuric acid synthesis and thus produces a negative result even with liver damage. Cholestasis and hepatocellular degeneration are not well differentiated, and in

prolonged obstruction very abnormal results have been found [3023]. In carcinoma metastases abnormal results are frequent, whereas in lymphomas they are not.

Cinchophen-oxidation Test

After the administration of a test dose of 0.45 gm cinchophen, the urinary excretion of oxy-cinchophen is determined by a color reaction, and the results are expressed as a percentage of the amount given [1996]. In liver damage the excretion is increased, since the breakdown of cinchophen is incomplete. In very severe hepatic insufficiency the oxidation is also impaired, and less than normal amounts are eliminated [1996]. In patients without jaundice, increased oxy-cinchophen excretion suggests liver damage. The test is not widely used.

Other Tests

Many other tests involving transformations have been described, but none is extensively used [477, 943, 1996]. These include the excretion of oxysantonin in bile and urine after administration of santonin, the excretion of sulfuric acid compounds after administration of indole, *p*-cresol, or phenol, and the excretion of glucuronates after administration of camphor, menthol, and sodium salicylate.

DYE-EXCRETION TESTS

Injected dyes are excreted in the bile or in the urine. The preferential pathway is determined by the physicochemical characteristics of the dye. The excretion in the bile depends upon the hepatic circulation, as well as upon the function of the hepatic cells. The Kupffer cells are apparently bypassed during the removal of most of the dyes in clinical use.

Several methods are available for measurement of disturbed hepatic function with the help of dyes.

1. Measurements of the disappearance of the dye from the blood by comparing its blood concentration after mixing with that after a given period of time, and expressing the results as a percentage of dye remaining in the circulation. Clearance techniques using mathematical equations and the percentage disappearance rate have been recommended as a more exact means of expressing results [613, 1914].

2. Determination of the appearance time as

well as of the concentration of intravenously injected dye in the bile.

3. Determination of the concentration in the urine as an indication of a compensatory pathway in the presence of a damaged liver [503].

Bromsulphalein-retention Test

Physiologic Basis. Sodium phenoltetrabromophthalein sulfonate (Bromsulphalein, or BSP) is taken up by the hepatic cells and excreted in the bile. None can be demonstrated in ascitic fluid, and little is removed by the extrahepatic tissues. The rate of disappearance of BSP from the blood indicates the activity of the liver, and determinations of biliary BSP are used chiefly for physiologic studies. Under normal circumstances the disappearance rate from the blood stream is constant. Bromsulphalein retention in some instances of liver disease is associated with a progressive decrease in the clearance or disappearance rate, because the capacity of the hepatic removal mechanism is reduced by liver damage. In other instances the clearance is low but constant, suggesting an impaired blood flow through the hepatic lobule, rather than hepatic-cell damage. Consequently the uptake by the hepatic cells depends not only on the functional capacity but also on the efficiency of the circulation. Exercise, standing, and fever increase BSP retention. The influence of circulation upon hepatic BSP uptake permits its use in the estimation of hepatic blood flow, or splanchnic blood flow, by measuring the hepatic extraction by hepatic vein catheterization.

Methods. Originally, 2.0 mg per kg BSP was injected intravenously [2831]. The disappearance was determined by comparing the concentration 30 minutes after injection with that immediately after complete mixing of the dye in the blood stream. Results were recorded as percentage retention, the first specimen drawn being considered 100 per cent. Subsequently a solution containing 4.0 mg per 100 ml was taken as the 100 per cent standard, based on the fact that the plasma volume averages 50 ml per kg body weight. This eliminated the necessity of a sample immediately after mixing. Since changes of plasma volume are ignored with this standard, readings more logically should be recorded in terms of concentration, rather than as per cent retention.

The examination of several samples with the plotting of an excretion curve may reveal mild hepatic dysfunction causing only a temporary delay in clearance [1223, 1224, 2115, 2241]. Al-

though this is theoretically more sound, it is not required for practical purposes, for which only one sample is taken.

Agreement is lacking on the optimal dose and time of sampling. The following have been suggested: 2.0 ml per kg dose, with readings at 20 minutes [2240] or 30 minutes [2064], a 5.0 mg per kg dose, with readings at 15 [2362], 30 [1453], and 45 minutes [2242], and an intermediate dose of 4.0 mg per kg. The smaller dose has the advantage of a zero reading in normal cases, and it reduces any possible effect of an enterohepatic circulation. The 5.0 mg per kg dose is most widely used because of the increased burden on the liver and improved chances of testing its ability [2115]. A 4.0 mg per kg dose with readings after 45 minutes is probably optimal, in that the disappearance curve is linear under normal circumstances.

Spectrophotometric readings eliminate much of the interference due to blood and bile pigments. The BSP test can be combined with galactose- and hippuric acid-tolerance tests [604, 3709].

Bromsulphalein is less toxic than most other dyes, but rare reactions are seen, apparently anaphylactoid in nature, especially if serial tests are performed [540, 2343, 2837]. These reactions are best avoided by slow injection of the dye. Thrombophlebitis at the injection site may occur, especially if dye is infiltrated into the tissues.

Technique. The dye is supplied by Hynson, Wescott, and Dunning as a 5.0 per cent solution (0.1 ml contains 5 mg). The amount needed is calculated as follows. The body weight in pounds divided by 22 equals the volume of dye to be injected when a 5.0 mg per kg dose is desired. Some investigators recommend that obese persons should not receive more than 8.0 ml, since their liver size is not a function of the body weight. Slowly inject the dye intravenously, the first 50 mg within the first minute and the rest in the next 2 minutes, with care to prevent extravasation. After exactly 45 minutes, draw approximately 5.0 ml blood from the opposite arm and place in a test tube. After coagulation, separate the serum by centrifugation. Dilute 2 ml serum with 8.0 ml of 0.85 per cent saline solution. To 5.0 ml of the mixture, add 0.2 ml of 10 per cent sodium hydroxide solution, and to the remainder add the same amount of 10 per cent hydrochloric acid. Read the alkaline tube in the spectrophotometer at a wavelength of 580, using the acid tube as a blank. Read results on a standard curve prepared with known amounts of dye. Simple color comparators are available, furnished by

the manufacturers of the dye. Corrections are made in jaundice using serum blanks [3706].

Results. In normal persons, using any of the methods, the retention varies between 0 and 6 per cent. Abnormal values in hospital control patients occur less frequently than with almost any other test, and the test can be used in children [2328]. In hepatitis, cirrhosis, and biliary obstruction, abnormal results are found with great regularity (Table 42). Whether biliary obstruction

Table 42 Bromsulphalein Retention in Hepatobiliary Diseases

<i>Diagnosis</i>	<i>No. cases</i>	<i>Mean, %</i>	<i>Range, %</i>	<i>% abnormal</i>
Normal.....	145	0-6	1.4
Acute hepatitis.....	328	33.6	7.0-62	82.8
Cirrhosis.....	398	21.1	2.5-34	87.2
Extrahepatic biliary obstruction.....	79	15.0	4.0-29	78.7
Hepatic tumor metastases.....	104	17.0	3.0-50	92.7
Chronic passive congestion.....	106	5.0-100	85.0

Sources: Method—Rosenthal and White [2831]; data—Felder *et al.* [990], Mendelsohn and Bodansky [2267], Popper and Schaffner [2640], unpublished data [3394].

with regurgitation or inability of the hepatic cells to extract the dye from the plasma is responsible for retention can not be decided. This eliminates the use of the test in the differential diagnosis of jaundice.

HEPATIC-CELL DAMAGE. Bromsulphalein retention is one of the most sensitive tests for the recognition of hepatic-cell damage and can be used for screening purposes after exposure to hepatotoxic drugs, or during epidemics of hepatitis, or for the evaluation of hepatic function during recovery from hepatitis [156]. It is the first test to show abnormal results in early viral hepatitis [1420]. In cirrhosis without jaundice significant retention is often found, permitting differentiation from other types of hepatomegaly. The use of the test has also been suggested to differentiate massive gastrointestinal hemorrhage caused by esophageal varices from other sources of bleeding [3689]. In fatty livers without cirrhosis, BSP retention is usually high.

In incomplete biliary obstruction, BSP retention is out of proportion to the hyperbilirubinemia if secondary hepatic-cell damage is present, particularly in cholelithiasis or choledocholithiasis. Near-normal clearance in the presence of non-hemolytic jaundice suggests an uncomplicated common duct stone. If dye retention persists longer than other laboratory signs of biliary obstruction after relief of obstruction, residual hepatic-cell damage is present [482]. Even in acute cholecystitis without jaundice, dye retention is often noted [443].

NONHEPATIC DISORDERS. Bromsulphalein retention may be elevated in thyrotoxicosis, diabetes mellitus [2622] or febrile states [1482], malaria [2121], pneumonia, and other infectious diseases (Table 43). Abnormal retention often occurs in

Table 43 Diagnostic Significance of Bromsulphalein Retention

- I. Hepatic diseases associated with Bromsulphalein retention
 - A. Cirrhosis
 - B. Hepatitis
 - C. Cholestasis
 - D. Carcinoma
 - E. Fatty liver
- II. Extrahepatic conditions associated with Bromsulphalein retention
 - A. Congestive heart failure
 - B. Fever
 - C. Malaria
 - D. Infections
 - E. Shock
 - F. Gallbladder disease
- III. Hepatic diseases in which Bromsulphalein retention may be the only laboratory clue to diagnosis
 - A. Cirrhosis without jaundice
 - B. Anicteric hepatitis
 - C. Fatty liver
- IV. Diffuse hepatic disease in which Bromsulphalein retention is frequently not increased
 - A. Intermittent, incomplete extrahepatic cholestasis (stone)

hepatic carcinoma metastases [2267, 2543, 2944], when it is usually associated with increased serum-alkaline phosphatase activity [3031].

CIRCULATORY INSUFFICIENCY. The importance of hepatic circulation explains the abnormal results in congestive heart failure [990] or shock [734] without significant hepatocellular damage, or the very abnormal results if mild hepatocellular insufficiency is complicated by circulatory defects. This may also explain some of the increase in BSP retention after surgery [3290]. Similarly, the

reduced BSP extraction in premature infants and its improvement with maturation have been related to the gradual development of hepatic circulation [2470].

PROLONGED RETENTION. Determination of the BSP level in serum several days after the administration of the test dose indicates that the BSP retention persists much longer in obstructive jaundice than in acute hepatitis and cirrhosis [1163]. In cirrhosis the retention usually lasts less than 1 day and may have diagnostic significance.

EXPERIMENTAL ANIMALS. Bromsulphalein retention occurs in dogs with biliary fistulas [835] or with carbon tetrachloride intoxication [1234], and in rats with experimental fatty liver [1820], bromobenzene intoxication [1823], ligated common bile ducts [1824], or nutritional liver damage [1821]. The test can also be used in mice [510].

Hepatic Clearance or Extraction of Bromsulphalein

Hepatic BSP clearance represents the amount of blood cleared of BSP by the liver in any unit of time; the hepatic extraction ratio is the amount of BSP removed by the liver, also in any unit of time. Both quantities depend on the plasma volume. Clearances are calculated from data obtained from peripheral blood with the help of mathematical formulas [1223, 1914, 2265, 2403] and based on the assumption that BSP is removed only by the liver and that the disappearance rate follows a simple logarithmic curve. Since these assumptions may not necessarily be valid, the use of the term "clearance" is possibly not justified. The BSP extraction ratio requires examination of hepatic vein blood obtained by catheterization [1587, 3043]. Although theoretically ideal, it is too difficult for practical purposes. This technique has been used for the estimation of the hepatic blood flow (see Total Hepatic Blood Flow, Chap. 18).

Biliary Excretion of Bromsulphalein

Determination of BSP in duodenal fluid, with or without simultaneous determination in the blood, may give added information [482, 2909, 3634]. With the 2.0 mg per kg dose of BSP, dye appears in the bile within 15 minutes. In the first 2 hours, 50 to 85 per cent is excreted in the bile, and most of the rest appears in the next 2 hours. Biliary excretion thus lags somewhat behind blood clearance. All BSP can not be recovered in

the feces, because some is destroyed by intestinal bacteria [1165]. The method is valuable in mild conditions, such as incipient biliary obstruction or slight hepatocellular degeneration, where the biliary excretion is delayed despite normal blood clearance. Bromsulphalein excretion can also be used to differentiate gallbladder bile from liver bile [2909].

Evaluation. The simple BSP-retention test yields results almost equivalent to those of more complex methods and is therefore preferred for clinical use. Bromsulphalein retention reflects hepatocellular degeneration, cholestasis, and impaired circulation through the hepatic parenchyma. It is very sensitive for the recognition of hepatic-cell damage, especially without jaundice. It is used for screening for hepatic disease and for detection of cirrhosis, carcinoma metastases, or persistent hepatic-cell damage during recovery from hepatitis or after relief of obstruction. While it is one of the most valuable tests in the absence of jaundice, it has very little value in the differentiation of "surgical" from "medical" jaundice.

Rose Bengal Test

This test has been widely replaced by the Bromsulphalein test, although the original method has been made more reliable by the spectrophotometer. A standard dose is injected; blood samples are usually drawn after 2, 8, and 16 minutes. With intact liver function, the blood contains about 50 per cent of the 2-minute concentration after 8 minutes, and about 30 per cent after 16 minutes. Jaundice and hemolysis do not interfere with the determinations [1606]. The dye

rapidly accumulates in the bile [1005]. The test has been used in animals [3270]. In man, the danger of photosensitivity is a disadvantage. In addition, the short interval between venipunctures makes the test more susceptible to errors. The Bromsulphalein test is therefore preferable, especially since the interpretation of the results is identical and both tests are of equally limited use in jaundice. These two dye tests can be performed together [605], although Bromsulphalein inhibits removal of rose bengal [605].

Azorubin S Test

The excretion of azorubin S into the bile after administration intravenously is measured. A scarlet-red color normally appears in the duodenal fluid within 15 to 20 minutes after injection of 4.0 ml of a 1.0 per cent aqueous azorubin solution and subsequent installation of 40 ml of a 25 per cent magnesium sulfate solution into the duodenal tube [2815]. Delayed appearance or absence of the color signifies hepatic damage [943, 2815]. The test is without value in the absence of bilirubin excretion in the bile. Delayed appearance indicates persistent liver damage in cholelithiasis after biliary excretion has been restored. This usually coincides with hyperchromia of the bile. At present, the only use of the test is as a supplement to a duodenal intubation carried out for other reasons.

The other dye-excretion tests, such as those based on urinary excretion of phenoltetrachlorophthalein or phenolphthalein, have only historical interest, primarily because x-ray visualization of the gallbladder was an outgrowth of these tests.

ROENTGENOLOGIC VISUALIZATION OF THE LIVER AND BILIARY TRACT AND DUODENAL DRAINAGE

Cholecystography

Physiologic Basis. Excretion of phthalein dyes by the liver is the basis of cholecystography. Some phthaleins, such as phenolsulfonphthalein (PSP), are preferentially excreted in the urine, but the halogenated phenolphthaleins are primarily excreted by the liver in a conjugated form. Since tetraiodophenolphthalein is radiopaque, it was utilized by Graham and Cole for the roentgenologic visualization of the gallbladder [1243]. The original dyes have been replaced by better tolerated and less toxic ones. The amount of dye excreted after standard doses is usually insufficient to cast a shadow of the bile ducts. The removal of water from the bile by the intact gallbladder concentrates the dye to such a degree that a contrast shadow can be obtained after 14 hours. After a cholagogic substance has been administered, the gallbladder gradually contracts, and the shadow starts diminishing in size. Subsequently the gallbladder relaxes, and the remaining dye becomes diluted as the gallbladder is refilled during the period when the sphincter of Oddi is contracted. This decreases the density of the shadow, so that the gallbladder is no longer visualized. Sometimes the gallbladder refills owing to reabsorption from the intestine in an enterohepatic circulation.

Methods. The tetraiodophenolphthalein originally used has been replaced by β -(4-hydroxyl-3,5-diiodophenyl)- α -phenyl propionic acid (iodoalphionic acid, Priodax) [808, 900, 1229] with 51.5 per cent iodine, α -ethyl- β -(2,4,6-triiodo-3-hydroxyphenyl) propionic acid (iodophenoxic acid, Teridax) with 66.5 per cent iodine [3539] or 3-(3-amino-2,4,6-triiodophenyl)-2 ethyl propanoic acid (iopanoic acid, Telepaque) with 66.7

per cent iodine [2075] and a higher degree of biliary excretion [2956]. Telepaque produces the most dense shadow, but it also leaves an opaque residue in the intestine [2983]. As a rule, 3 gm in six $\frac{1}{2}$ -gm tablets is given at one dose. The patient should be kept on a fat-free diet from the time of the first dose of the dye, to avoid premature gallbladder reflexes. To overcome insufficient dye concentration in the gallbladder, a double dose of dye is often preferred [2225]. The first dose is given in the afternoon and the second after the evening meal, with subsequent roentgenologic examination the next morning 14 hours after the second dose. Prior to the administration of the dye, a scout film of the right upper quadrant is taken. This in itself may yield valuable clinical information [3219]. The position of the patient during the examination is a technical roentgenologic problem [1775, 3140]; the gallbladder varies greatly in its location. Pitressin has been given in order to remove gas or fecal shadows in the colon if these interfere with good visualization. The gallbladder dye also can be injected intravenously, but this procedure had been abandoned largely until recently because of the simplicity of oral administration. The dye was given slowly, and 12 to 14 hours were allowed to elapse before roentgenograms were taken. The excretion was often accelerated by giving Decholin or hypertonic glucose solution with the dye.

The motility of the gallbladder is tested by comparing the size of the gallbladder before and after the intake of strongly cholekinetic material, such as a mixture of egg yolk and cream (Fig. 42). The diminution in gallbladder size after the fat meals is mainly caused by evacuation, although selective absorption has also been held partly responsible [1361].

Results. Cholecystography provides information about position, size, shape, contour, and motility of the gallbladder. The contraction phase also helps to visualize stones or circumscribed lesions of the wall, e.g., papillomas, which may not show on standard films.

CONTRAST VISUALIZATION. The contrast visualization of the gallbladder depends upon (1) hepatic function in excreting the dye; (2) patency of the hepatic and cystic ducts; (3) concentrating ability of the gallbladder mucosa. A faint shadow may result from impaired excretion, or, more frequently, from reduced concentrating ability of the gallbladder wall as in acute inflammation, or from other changes of the gallbladder mucosa, including tumors. Technical errors should also be considered. Filling defects within the contrast shadow of the gallbladder suggest stones. Radiopaque stones appear on the scout film, but radiolucent stones are demonstrable as filling defects. They may be more apparent in the erect position and may be associated with a layer of translucency above the contrast shadow in the fundus. Occasionally the intensity of the contrast shadow prevents the demonstration of small stones or gravel. Benign papillomatous tumors can be visualized as marginal defects retaining their marginal site during evacuation after a fat meal [1774].

VARIATIONS IN SIZE, SHAPE, AND CONTOUR. Many variations are related to the body habitus of the patient, e.g., elongation or ptosis, the latter occasionally associated with hepatoptosis. Deformity of the gallbladder shadow may be caused by pericholecystic adhesions, congenital variations such as the phrygian cap, or, rarely, external compression. Tentlike distortions resulting from adhesions often become apparent after a fat meal.

MOTILITY. Examination of the motor action of the gallbladder, or a motility test, is an essential part of cholecystography. Films taken at various intervals after a fatty or egg-nog meal show a gradual diminution in the size of the shadow, indicating the ability of the gallbladder to contract adequately. Normally the shadow is approximately half its original size after 30 to 45 minutes. Delayed contraction is said to suggest biliary dyskinesia and not necessarily organic changes. A dense shadow generally rules out major anatomic changes of the gallbladder. An associated delayed emptying has many causes, such as spasm of the sphincter of Oddi, hypomotility or atony of the gallbladder, or poor response re-

sulting from dietary or metabolic causes, such as starvation, high-carbohydrate diet, lipemia, or pregnancy. Hypomotility is usually associated with a large gallbladder. Hypertonic dyskinesia owing to spasm of the sphincter of Oddi may be recognized by pains occurring after the fat meal.

NONVISUALIZATION. Nonvisualization of the gallbladder, often confusingly called a "positive Graham-Cole test," has several causes.

1. Faulty intestinal absorption of the dye because of diarrhea, pyloric obstruction, or other gastrointestinal diseases.

2. Technical errors in visualization or abnormal position of the gallbladder.

3. Decreased excretory ability of the liver. This last occurs in many hepatic disorders and, as with the excretion of any dye, circulatory efficiency is also important. Inability to excrete gallbladder dye is not necessarily related to jaundice, and in recovery from hepatitis, visualization can often be obtained while jaundice still exists. In acute hepatitis administration of a double dose of dye supposedly results in visualization of the gallbladder in 90 per cent of patients with serum-bilirubin levels below 11 mg per 100 ml [2724]. Nonvisualization is common in cirrhosis or hyperthyroidism even in the absence of jaundice. Faulty excretion by the liver is usually associated with excretion of the dye in the urine.

4. Obstruction of the common and hepatic ducts. In biliary obstruction the cholestasis prevents filling of the gallbladder by dye. If the obstruction is at the papilla of Vater and if the hepatic and cystic ducts are patent, small amounts of dye accumulate in the gallbladder, and after 2 or 3 days a very distended gallbladder may be faintly visualized, a roentgenologic Courvoisier sign.

5. Obstruction or inflammation of the cystic duct. Inflammation, strictures, or calculi within the cystic duct, as well as hypertrophy and enlargement of the gallbladder, prevent the filling of the organ. A gallbladder full of stones or mucus and a gallbladder neck choked with stones have the same effect.

6. Loss of the concentrating ability of the gallbladder wall. Inflammatory and fibrosing lesions of the gallbladder mucosa reduce the concentrating ability of the gallbladder. Faint visualization or nonvisualization of the gallbladder may thus be a sign of inflammation. In chronic cholecystitis, the concentrating ability is sometimes eventually restored by secondary hypertrophy of

the mucosa. Nonvisualization also occurs if the patient is on a fat-free diet for several days, because the gallbladder is distended with viscid bile [701].

7. Premature gallbladder reflex. If a gallbladder contraction occurs in the interval between the administration of the dye and the roentgenologic examination, the dye-containing bile may have been expelled from the gallbladder. Proper technique of cholecystography is aimed at avoiding such untimely gallbladder contractions, especially by administration of nonchologogic food prior to examination [2789]. In some cases, particularly in the presence of emotional factors or of diseases that increase gallbladder irritability, such as peptic ulcers [2037], an untimely gallbladder reflex can not be prevented.

Evaluation. Roentgenologic visualization of the gallbladder is improving with the development of new dyes, so that lack of visualization can be attributed without question to abnormalities of the liver or biliary tract. Visualization can not be expected with any degree of certainty in jaundice of nonhemolytic origin, since in hepatocellular degeneration the liver excretes the dye poorly and in cholestasis the dye does not reach the gallbladder. Cholecystography is helpful in jaundice mainly in the defervescent stage and is usually not undertaken if the total serum bilirubin exceeds 3.0 mg per 100 ml.

Direct Cholangiography

In recent years direct cholangiography has been developed, the ducts being filled with radiopaque material either during operative exposure or through an external biliary fistula, if such is present. Cholangiography permits visualization of the entire biliary system, which is possible only occasionally in routine cholecystography if the picture happens to be taken during the period when the radiopaque bile passes through the common duct. Operative cholangiography is performed during surgery or later by injecting the opaque material into the drainage tube [202, 262, 508, 1478, 2603]. The material must be sterile, and the injection pressure should be low [2320]. The risk is minimal [434], and serious consequences such as pancreatitis have rarely been seen. Injected radiopaque material normally enters the duodenum. With mechanical or spastic obstruction, filling of the dilated intrahepatic biliary tree is noted (Fig. 82). Functional obstructions are generally transient, and serial

roentgenograms are recommended in such cases. The intrahepatic tree is often visualized even in the absence of obstruction [967], and the pancreatic duct is also often filled (Fig. 42).

The gallbladder has been injected directly under peritoneoscopic visualization after pneumoperitoneum has been induced [1441, 1711, 2843, 2845], or through a small abdominal incision. This technique is the most direct way of demonstrating the patency of the biliary system and is theoretically the best procedure in the differential diagnosis of hepatobiliary disease, especially in instances of puzzling jaundice [2845]. Biliary dyskinesia can also be demonstrated [2844], and pressure relations can be measured by radiomanometry [34]. However, the question of safety, the danger of leakage from the injection site in the gallbladder, and the possibility of stone formation have thus far prevented widespread application of the method.

Oral and Intravenous Cholangiography

In the attempt to demonstrate the biliary system in the absence of the gallbladder or in instances of nonvisualization, large doses of Telepaque combined with administration of paregoric and dehydration of the patient have been recommended [3379]. When visualization occurs, it is usually very faint.

Recently Cholografin, or Biligrafin, the disodium salt of N,N'-adipyl-bis(3-amino-2:4:6-triiodobenzoic acid, or sodium iodipamide), which opacifies the bile ducts after intravenous administration, has been introduced [1115, 2675]. The compound contains 64 per cent iodine and is relatively non-toxic. Even better visualization has been obtained by using either the lithium salt of iodipamide or iodipamide methylglucamine. Transient side effects such as restlessness, nausea, or vomiting occur if the drug is given too rapidly. The usual dose of 40 ml should be given over a period of 10 minutes after conjunctival or intradermal sensitivity tests. If iodipamide methylglucamine is used, the dose is only 20 ml. Immediately following the injection, films are taken; they are repeated every 10 minutes for 1 hour and are taken again 2 hours after the injection. If liver function is impaired, the dye is excreted by the kidneys and a pyelogram is seen instead of a cholangiogram. Intravenous cholangiography should be used if patients who had a prior cholecystectomy have symptoms referable to the biliary system, if the gallbladder fails to visualize after oral dye,

or if the patient is unable to retain oral dye [3034]. The extrahepatic ducts have been visualized with a high degree of regularity in patients whose gallbladders have been removed. Nonopaque stones are also well visualized. Sometimes the opacification of the duct is unexplainedly very faint, and the terminal portion of the duct usually can not be seen distinctly. When the serum-bilirubin level is above 4.0 mg per 100 ml, or if Bromsulphalein retention is greater than 20 per cent, the ducts are usually not visualized. The use of the procedure may prove invaluable in differentiating intrahepatic from extrahepatic cholestasis with slight jaundice.

Hepatosplenoportography

Various contrast media have been used in attempts to outline the liver and spleen roentgenologically. Pneumoperitoneum and filling the stomach and colon with air or gas [3700] have yielded limited information on the size and position of these organs. Colloidal thorium dioxide, or Thorotrast, which is deposited in the reticulo-endothelial cells of the liver and spleen following intravenous injection, has also been used. Because of the deleterious effects of the radioactive material, its use for this purpose has been abandoned. Recently iodine-containing dyes, such as Diodrast, have been injected percutaneously into the spleen to outline the portal vasculature [651, 2339, 2840]. This has resulted in visualization of the portal vein and also of the coronary vein in portal hypertension and of nodules in the liver. The procedure has been recommended for all patients prior to shunt surgery, to aid in deciding the type of shunt to be made [1628]. The dye has also been injected directly into the liver. Patients must be tested for sensitivity to the dye, either intradermally or conjunctivally, prior to its injection. Severe pain, splenic lacerations, or catastrophic idiosyncrasy has occurred following this procedure.

Other Radiologic Procedures

Flat films of the abdomen, barium meals, and barium enemas have been used in the diagnosis of hepatobiliary diseases. Visualization of the gastrointestinal tract sometimes assists in locating the gallbladder and in demonstrating internal biliary fistulas and lesions of the ampulla of Vater or of the pancreas. A barium swallow is indicated in every patient with chronic hepatic disease to detect the presence of esophageal varices [2603].

The finding of varices is occasionally the first indication of chronic hepatic disease.

DUODENAL DRAINAGE

Examination of the duodenal contents obtained by intubation provides information as to normal and pathologic physiology of the biliary tract and provides clues as to hepatic function. Technical difficulties and the inconvenience to the patient have limited its use. Those who have acquired wide experience with this procedure have been enthusiastic about its application [324, 1835].

Procedure. A thin duodenal tube, 10 to 14 F and 130 cm long, with or without a metal tip, is introduced into the stomach and the stomach contents aspirated. The patient is then placed on the right side, and the tube is slowly advanced while the patient is well relaxed. When light-yellow or golden-brown alkaline fluid is obtained, the tube is in the duodenum. The stomach contents, in contrast, are acid and turbid and contain mucin. Fluoroscopy is helpful in localization. Fluid instilled into the stomach can be almost completely recovered by aspiration; this is not true in the duodenum. The tube sometimes does not readily enter the duodenum. If it is coiled up in the stomach, the tube has to be withdrawn. If hyperacidity is present, warm bicarbonate solution should be instilled. The duodenal content without bile is light yellow and clear. Entrance of the tube into the duodenum often relaxes the sphincter of Oddi, and bile is then released into the duodenum. Subsequently 25 ml warm saturated solution of magnesium sulfate is instilled into the tube, which is then clamped for 2 minutes; the magnesium sulfate is then permitted to drain. A continuous flow of a golden-brown bile, derived from the intrahepatic and extrahepatic biliary ducts, begins. It represents freshly secreted liver bile mixed with bile in the ducts. After several minutes, when approximately 10 to 30 ml of A bile has been collected, about 30 to 60 ml of a darker-green viscid bile, B bile, appears. The darker B bile is probably gallbladder bile expelled after contraction of this organ, although this has been questioned, since dark B bile has been collected after cholecystectomy. If B bile does not appear upon magnesium sulfate stimulation, 5 to 10 ml olive oil dispersed in water is instilled and the tube is clamped for 10 minutes. After all B bile has drained, the bile again becomes light, C bile;

this is unmixed liver bile. All three fractions should be examined. Liver bile can also be differentiated from gallbladder bile with colored dyes or with the iodine-containing dyes used in cholecystography [1346].

Inspection of the Bile. Absence of bile pigment from the duodenum in the presence of a correctly located tube is a reliable means of establishing biliary obstruction. Absence of B bile indicates either obstruction of the cystic duct, or atony, or faulty concentration by the gallbladder. The presence of blood suggests a carcinoma at or near the papilla of Vater, or a duodenal ulcer. Following intravenous injection of azorubin S, the bile quickly becomes red, normally (see Azorubin S Test, Chap. 37). This reaction is delayed or does not take place in the recovery period after extrahepatic biliary obstruction or in other hepatic injuries, even if bile pigment excretion in the bile is normal.

Microscopic Examination. Drops aspirated from the bottom of the collecting tube are examined unstained and without centrifugation. The presence of segmented leukocytes suggests gastritis, duodenitis, cholangitis, or cholecystitis.

Leukocytes and epithelial debris may be present in any of the three bile fractions. Bile staining of leukocytes indicates their origin from the biliary tract. In empyema of the gallbladder, large amounts of pus cells are found exclusively in B bile; in cholangitis they are found in C bile. Cholesterol crystals are flat rhomboid plates with chipped edges. Calcium bilirubin crystals are lustrous granular clusters. They indicate a tendency for precipitation and are found in cholelithiasis or choledocholithiasis [1575]. Application of the methods of exfoliative cytology to the duodenal contents has been recommended for the early diagnosis of malignant lesions of the papilla of Vater, the biliary tract, and the pancreas [1947]. The diagnostic accuracy is somewhat greater than in the study of gastric contents for carcinoma of the stomach [1947]. The collected duodenal contents are cooled with ice and rapidly centrifuged; the residue is smeared and stained.

Microbiologic Examination. The presence of bacteria in the gallbladder or biliary tract and the typhoid carrier state can be established by examination of bile, although careful sterile methods have to be applied to avoid contamination. *Giardia lamblia*, *Trichomonas hominis*, *Ascaris lumbricoides*, and even *Endamoeba histolytica* have been found in the duodenal fluid.

Chemical Examination. The bile pigment concentration can be estimated with the icteric index. The difference in pigment concentration between B and C bile is a measure of the concentrating ability of the gallbladder. In hemolytic jaundice, C bile is dark and resembles B bile. Also, after cholecystectomy, the liver bile contains more bilirubin than normal. Urobilinogen is found only in small amounts in the bile normally, but in biliary tract infections, *d*-urobilinogen has been found [2080]. In hemolytic jaundice, urobilinogen may be present in bile because of increased absorption from the intestine and reexcretion as such by the liver. Increased biliary urobilinogen in hepatocellular degeneration has been explained by inability of the liver to reconvert it to bilirubin [943].

The determination of either the total bile acid or individual bile acids in bile is difficult, and no satisfactory methods are available for the clinical laboratory. Protein has been found in bile, possibly as a result of hepatic-cell damage. The demonstration of tryptic or other enzymatic activity in the duodenal contents helps to evaluate pancreatic function. These enzymes are reduced or absent in carcinoma of the head of the pancreas or in pancreatic lithiasis.

Evaluation. Duodenal drainage offers much information as to the functional state of the liver and biliary tract. However, the recent emphasis on roentgenologic examination of the gallbladder and upon the simpler laboratory tests has discouraged the use of this technique, which places a strain on physician and patient. With experience, these disadvantages can be gradually decreased. Nevertheless, few studies based on duodenal drainage have appeared in recent years, except for those concerning exfoliative cytology.

The histologic examination of specimens of liver tissue obtained at laparotomy has long been an established procedure. Biopsy with a needle as a widely used routine diagnostic procedure is a recent development. Needle puncture of the liver had been performed repeatedly during the nineteenth century and the first third of this century, primarily for drainage of hepatic abscesses and hydatid cysts. Reviews of the early studies emphasize the relatively high mortality rate [164, 745, 1518, 1855]. In 1939, systematic studies of material removed for examination were reported from the United States [164]; at approximately the same time, similar studies were reported from Denmark [1601]. Somewhat later, German [125] and British [786, 3040] reports appeared. During World War II wide use of liver biopsies was started with the needle alone or with the peritoneoscope. Despite the risks, the method now appears to be a well-established diagnostic procedure, and a large volume of literature is available concerning technique, contraindications, diagnostic results, and academic studies.

METHODS

Liver biopsies can be obtained by three different means: (1) at the time of abdominal operation; (2) with the aid of the peritoneoscope; (3) through the intact abdominal or chest wall with a needle.

Surgical Biopsy

Laparotomy is rarely performed solely for the purpose of obtaining a specimen of liver, but small pieces of liver have frequently been excised during operations on the biliary tract, stomach, or duodenum [1243, 2279]. Much information as to

the general physiology and pathology of the liver has been obtained from such biopsies and the diagnosis has been established in many instances. One drawback of this method is the fact that the subcapsular portion usually obtained is not representative of the hepatic tissue as a whole, because fibrotic changes often associated with bile duct proliferation are present. If the biopsy is taken from the inferior edge of the liver, as is commonly done, a picture of cirrhosis may be seen in essentially normal livers, especially in people over twelve years of age. Specimens from the gallbladder bed are even less desirable. As a routine diagnostic method, surgical biopsy is extremely limited, despite the facts that the site of the biopsy can be selected, the danger of hemorrhage can be minimized, and large specimens can be obtained, permitting the use of several fixatives.

Peritoneoscopic Biopsy

With the peritoneoscope, biopsies can be obtained from well-visualized sites of the liver, either with a biopsy forceps [1441, 1712, 2120, 3452] or with a needle [1518, 1678, 2548]. Peritoneoscopic biopsy requires experience and training in peritoneoscopy, as well as elaborate equipment which is often not readily available. Otherwise this method has the following advantages:

1. The biopsy site can be visualized, which is especially important in focal lesions, such as tumor metastases.
2. The histologic findings can be correlated with the gross appearance.
3. The specimens obtained by forceps are fairly large; this is important in lesions which do not produce uniform changes.
4. Several pieces can be obtained without difficulty for several fixatives.

5. Hemorrhage is easily recognized and treated, and larger vessels are avoided, although fatal bleeding has been reported [1441].

The major disadvantage of forceps biopsy is the same as that of surgical biopsy; namely, the subcapsular changes are misleading.

Needle Biopsy

Biopsy Sites. For liver biopsy by needle inserted blindly through the skin, three sites are used: (1) anteriorly below the costal arch [164, 745] (Fig. 63); (2) laterally through the ninth intercostal space [1601]; (3) medially at the costoxiphoster-nal angle [1172]. This last method was advocated to reach the left lobe of the liver and to reduce the chances of piercing larger vessels and the danger from respiratory movements. It found little acceptance, primarily because of the difficulty in percussing the borders of the liver in this location [3313]. The anterior subcostal approach can be used only with large livers, and the danger of laceration, probably because of greater respiratory excursions, seems to be higher with this method, judging from the number of reported accidents [3313]. Therefore, this method is recommended only to reach palpable nodules in the presence of pulmonary or pleural disease, and in the presence of extremely narrow intercostal spaces to avoid laceration of the intercostal artery. This approach is also suggested in small children [428]. Otherwise, the lateral approach is recommended, since it best permits percussion of the liver dullness, and since the respiratory excursions are relatively small.

Needles. The specimen is removed by an aspirating syringe attached to the needle, or by the cutting action of the needle itself.

ASPIRATION NEEDLES. The original Iversen-Roholm needle is a simple trocar, consisting of a canula with a sharp-ground edge and a pointed stylet [1601]. This needle is inserted into the liver, the stylet removed, a syringe attached, and the canula advanced and then withdrawn while suction is applied. The Franseen needle consists of a 14-gauge needle with an obturator with three bevels at the tip instead of one [1078]. It is used somewhat like the Iversen-Roholm needle, its advantages being a better cutting edge and a Luer-Lok hub. The Roth-Turkel needle [2836] consists of an outer trocar and stylet which is inserted to the surface of the liver [745]. The stylet is then withdrawn, and a hollow inner needle with a saw-toothed end is inserted. This is advanced and

rotated. Then a syringe is applied, and the needle is withdrawn as suction is applied. The chief advantages of this method are that the specimen is fairly large and that little or no distortion of tissue occurs [745]. The chief disadvantages of these aspiration techniques are the length of time required to remove the stylet and apply the syringe and the possibility that the trocar tip may leave a gaping hole in the liver capsule [1172]. Other trocars have been described [1468] but have not been widely used. The construction of one-piece syringe and needle instruments with beveled needles has best overcome these objections, and the Terry modification of the Gillman needle is therefore recommended [1172, 3313].

PUNCH NEEDLES. The simple and less expensive punch or cutting needle devised by Silverman [3073] found early [3360] and wide [602, 1518, 1771, 1815, 2372, 3350, 3382] acceptance in the United States. The needle consists of a long 14-gauge hollow-ground bevel-tipped outer portion and a slightly longer longitudinally split inner needle. Many modifications exist [593, 1518, 2424, 2891, 3073, 3427]; the most simple and effective is filling the tip of the inner needle with silver solder to ensure removal of the specimen after cutting [1070]. The chief disadvantages of the method are the small piece of liver removed, usually 1.0×15 mm, and distortion of the tissue by compression.

Technique. The procedure is best carried out in the patient's own bed, with the patient supine at the edge of the bed and his right hand under his head. Pentobarbital, 0.1 gm, should be given $\frac{1}{2}$ hour before the biopsy to allay apprehension. The area of liver dullness is percussed in the anterior or mid-axillary line. The interspace in the center of the dullness is the one used (Fig. 63). The area is swabbed with alcohol, and then a skin wheal is made with procaine at the site selected. The deeper tissues down to the diaphragm are infiltrated, and a 3-inch 20-gauge needle is used to infiltrate the diaphragm and liver capsule. The patient is instructed to breathe in and out and then to hold his breath in deep expiration. The needle is inserted to the hepatic capsule, and 3 to 5 ml procaine is injected as the needle is withdrawn. Care must be taken during the entire procedure to avoid the intercostal vessels, which are along the lower border of each rib. A small skin incision is made with a pointed scalpel blade. After the incision stops bleeding, the patient is ready for the biopsy. The punch biopsy with the Vim-Silverman needle and the aspiration biopsy using Terry's modification of the Gillman technique are described.

PUNCH BIOPSY. The stylet is removed from the Vim-Silverman needle, and the split inner needle is inserted in its place until the tip of the inner needle is visible at the bevel of the outer needle. It is then withdrawn so that it is just out of sight. The hub of the outer needle is grasped by the right thumb and index finger, and the needle is inserted through the incision to the diaphragm. The patient is then instructed to hyperventilate again and hold his breath in deep expiration. The needle is pushed through the diaphragm into the liver until the hub is about 1 inch from the skin. The inner split needle is advanced with the left hand as far as it will go. The outer needle is again advanced until the hub is almost at the level of the skin, while the left hand steadies the inner needle and prevents its advance. Both needles are then quickly withdrawn, the inner needle is removed from the outer one, and the piece of liver tissue which lies in one of the hollow halves is placed in the fixative. Twisting the needle is not necessary if the filled-tip inner needle is used.

ASPIRATION BIOPSY. A modified Gillman instrument with a 1.65-mm bore needle with a beveled knife edge and fitted stylet attached to a specially constructed syringe is used [3313]. The piston of the syringe is lubricated with mineral oil when the instrument is assembled. The syringe barrel is gripped in the right hand, while the needle is steadied by the left. The needle is inserted through the skin incision, and the patient is told to hold his breath. The needle is advanced until it is felt to be in the liver. It is withdrawn just out of the liver, the depth guard is adjusted, and the patient hyperventilates and again holds his breath in expiration. The point of the needle is readvanced about 1 cm into the liver. While the left hand holds the syringe plunger, preventing its forward motion, the needle is quickly advanced into the liver about 3.0 cm by thrusting the syringe barrel forward with the right hand. The entire syringe is then rotated and quickly withdrawn. The motions must be quick and precise, and the syringe plunger must remain motionless while the barrel is moved forward. The piece of liver tissue aspirated is gently extruded from the needle into a glass tube about 2 in. long with a 3.0-mm bore, in which it can be examined by transillumination. It is then placed in the fixative.

AFTERCARE OF THE PATIENT. A dressing is placed on the biopsy site, and the patient is instructed to remain recumbent for 6 to 8 hours and to remain in bed until the next morning. The patient's pulse is checked every 20 to 30 minutes for 2 hours and then hourly for the rest of the day. Various sedatives or analgesics are used to control pain if necessary. Tetraethylammonium chloride intravenously in 200-mg doses is said to best control pleural or diaphragmatic pain [2892]. Any drop in blood pressure or increase in the pulse rate should serve as alarm signals of hemorrhage. Usually transfusion of 1 to 2 pt blood

is all that is necessary to stop this. Surgical intervention should be considered if the patient goes quickly into shock or if a liter of blood does not seem to control the symptoms. The use of plugs of oxidized cellulose and thrombin has been suggested to prevent hemorrhage [593]. Signs of peritonitis, usually indicative of seepage from a bile duct, also call for exploration, with the possibility of relieving an extrahepatic biliary obstruction.

The biopsy wound quickly disappears. In a series of cases in which necropsies were performed 3 to 31 days after biopsy, no trace of the needle wound in the liver could be found, while in a few cases ex-

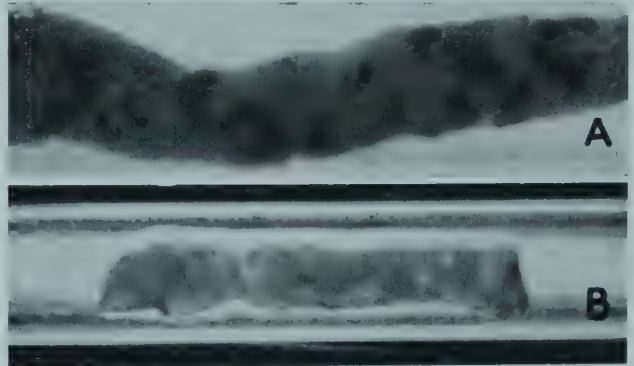


FIG. 130 A. Speckled biopsy specimen of liver obtained by the Terry needle from a patient with extrahepatic cholestasis. B. Transilluminated biopsy specimen showing translucent follicles of Boeck's sarcoid. (Terry, R. B.: *J.A.M.A.* 154:990, 1954.)

aminated $\frac{1}{2}$ to 9 days after biopsy, the stab wound was still seen, and in only three cases were remnants of bleeding found [2803].

GROSS INSPECTION OF THE SPECIMEN. The inspection of the aspirated specimen in a glass tube with transillumination permits recognition of granulomas and tubercles [3313] (Fig. 130). Cholestasis is readily recognized by the green discoloration of the centrilobular zones.

Indications, Contraindications, and Dangers

Although the risks of liver biopsy have greatly decreased in recent years, the procedure is not completely innocuous. Therefore, it should be used only if other studies do not yield the information desired, or if the histologic picture alone or in combination with the clinical and laboratory findings becomes a decisive factor.

Indications. The indications for liver biopsy are broadly outlined here; they should be qualified in the individual case, depending on the clinical picture and the laboratory findings (Table 44).

The indications depend upon the experience of the pathologist in interpreting the specimen and

upon the technique used. Knowledge of routine pathology and relatively small specimens suffice for the recognition of the cause of hepatomegaly

Table 44 Indications for Liver Biopsy

- I. In differential diagnosis of jaundice:
 - A. Disagreement between laboratory and clinical features
 - B. Persistent low-grade jaundice
 - C. Cholestasis of 3 weeks' or more duration, to differentiate intrahepatic and extrahepatic forms
- II. Hepatomegaly
- III. Nodular liver
- IV. Diagnosis of granulomatous disease
- V. Attempt to supply academic information
- VI. Follow-up of course and effect of therapy
- VII. Diagnostic problems with suspected hepatic involvement
- VIII. Clinical symptoms in posthepatic state (to prove presence of chronic hepatitis)

or of a nodular liver; for instance, for recognition of the presence of cirrhosis, fatty liver, amyloidosis, or diffuse tumor infiltration. In contrast, for the differential diagnosis of jaundice, special experience in the interpretation of liver biopsies is required. Many of the criteria used in this differential diagnosis are still in the process of clarification and classification. Similarly, for the follow-up of the course of a disease and for academic studies, familiarity with the histology of the liver in biopsy specimens is required.

Contraindications. The absolute contraindications to the use of liver biopsy are hypoprothrombinemia and hemorrhagic tendencies, the presence of infection, and the possibility of lacerating dilated aberrant bile ducts in the capsule in prolonged obstructive jaundice (Fig. 36, bottom) (Table 45). The relative contraindications vary

Table 45 Contraindications for Liver Biopsy

- I. Absolute
 - A. Hemorrhagic diathesis
 - 1. Hypoprothrombinemia
 - 2. Thrombocytopenia
 - B. Cholemia
 - C. Prolonged obstructive jaundice
 - D. Cholangitis and liver abscess
 - E. Infection in pleural cavity or right lower lobe of lung
- II. Relative
 - A. Congestive heart failure
 - B. Ascites
 - C. Hypertension
 - D. Anemia
 - E. Lack of cooperation on part of patient

and depend upon the condition of the individual patient. While passive congestion of the liver is usually regarded as a contraindication, some groups have performed biopsies as part of a study of the condition [3040, 3589].

Dangers. Complications and death resulting from liver biopsy have been repeatedly reviewed [359, 1120, 1518, 2640, 2803, 2893, 3313, 3690]. In recent years, however, the mortality rate is decreasing, while the number of biopsies reported is increasing. One of the latest reviews, consisting of over 20,000 biopsies, reports an incidence of 34 deaths, or a mortality of 0.17 per cent [3690]. This does not take into account the deaths after biopsies reported in studies before 1939. Doubtless some deaths have not been reported, and also in some of the reported deaths liver biopsy was not directly responsible, although it caused hemorrhages and preceded death.

The most common cause of death is hemoperitoneum as a result of laceration of liver tissue, usually of a vein. Hemorrhage occurs from the small liver biopsy opening without laceration if a hemorrhagic tendency with hypoprothrombinemia or thrombocytopenia is present. This underscores the importance of prothrombin determinations shortly before the biopsy and of thrombocyte counts in any condition in which thrombocytopenia may occur, such as leukemia or some forms of hypersplenism, although these measures have been disregarded in special cases without mishap [3591]. The bleeding may be rather insidious and not recognized clinically [2224]. Another cause of hemorrhage is laceration of an intercostal artery or of the cystic artery.

Deaths from biliary peritonitis following laceration of a subcapsular bile duct [1120], bile embolism because of a communication between a distended bile duct and the hepatic vein [413], or purulent peritonitis from perforation of a hepatic abscess [3313] are uncommon.

The nonfatal complications are similar to the fatal ones. The most important is hemorrhage from the liver or from the diaphragm usually into the peritoneal cavity and occasionally into the pleural cavity. Biliary peritonitis is produced mainly by perforation of bile ducts. Perforation of various organs with or without demonstration of renal, pancreatic, gallbladder, or intestinal tissue in the biopsy specimen has been reported [745, 1125, 3313]. When the right kidney is enlarged, particularly as a result of a cyst or abscess, care must be taken not to penetrate it.

Pulmonary complications such as pneumothorax [2372], pulmonary collapse [3440], or a friction rub occur [602]. Subcutaneous emphysema [3382], abdominal hematomas [3350], and subcutaneous bleeding [2372] have been reported. Pain in the shoulder, chest, flank, or abdomen occurs in as many as 30 per cent of all patients. The incidence of these complications varies [3313] and depends upon techniques used and the closeness of observation. The unique occurrence of carcinoma implantation in the needle track has been observed [3133].

APPLICATIONS OF LIVER BIOPSY

Statistical analysis of results has been reported by many investigators [234, 289, 1871, 2372, 2921, 3382]; the figures depend on the observer's indications for liver biopsy. When liver biopsy is done on a large scale, established clinical diagnoses are often confirmed and unsuspected conditions are detected. Nevertheless, even in very large series, the biopsy is of no help in almost 30 per cent of the cases [2921]. The usefulness of the procedure depends upon the adequacy of the specimen obtained and upon the diagnostic accuracy that can be achieved with the small sample of the liver.

Adequacy of Specimen. Complete failure of liver biopsy, or the obtaining of unusable fragments, occurs in 3 to 15 per cent of all biopsies [745, 2372, 2921, 3350, 3382, 3440]. This incidence decreases with experience. The adequacy of the usable specimen is relative to the purpose of the biopsy. For focal lesions such as tumors and granulomas a very small specimen may suffice if a characteristic portion is included in the specimen. For instance, tumor cells, tubercles, or parasites can readily be identified if present in small specimens. On the other hand, in these conditions the largest specimens possible are desirable in order to reduce the incidence of "false negative" biopsies. Nodules seen on gross inspection and not found in the first histologic section call for examination of serial sections. The minimum requirement for the recognition of diffuse hepatic disorders such as hepatitis or fatty metamorphosis is a cross section of at least two lobules with one or two portal tracts. For inflammatory lesions and cirrhosis, the biopsy specimen should be as large as possible to permit accuracy in diagnosis somewhat comparable to that with necropsy specimens. Systematic comparison of biopsy and autopsy

specimens shows that as far as hepatic-cell degeneration and fatty metamorphosis are concerned, the needle specimen is representative of the entire organ, while for alteration of the lobular pattern, scarring, and inflammatory changes, it is not necessarily so [1172, 3468].

In postnecrotic cirrhosis with large nodules, normal tissue can be found in the biopsy specimen if the core is obtained from the center of a nodule where the lobular architecture is intact. In specimens taken during surgery, focal necrosis and leukocytic infiltration are common artefacts [1715, 2244].

Diagnostic Accuracy. The diagnostic accuracy of liver biopsy can be ascertained by comparing the biopsy findings with laparotomy or necropsy findings obtained subsequently, or with the clinical diagnosis confirmed by the clinical course, or by the laboratory or operative findings [359, 745, 1125, 1525, 3350, 3457]. Diffuse lesions are misinterpreted only exceptionally. Cirrhosis is the diffuse lesion most often missed—in nearly 10 per cent of cases. Focal lesions are missed more often; for instance, 20 to 30 per cent of primary or metastatic carcinomas are missed on biopsy. Therefore a positive finding is highly significant, while a negative or normal specimen excludes diffuse diseases but not focal ones [3691].

Specific Problems Studied with Liver Biopsy

To illustrate the general applications of liver biopsy, a brief and necessarily incomplete summary is presented of observations referred to in detail in the respective chapters of the book.

Liver Biopsy in Hepatitis. The history of non-fatal viral hepatitis is largely based upon liver biopsy studies [125, 775, 786, 1714, 1872, 2189, 2801, 2802, 3541]. Criteria for diagnosis [2897] and for the differentiation of viral from toxic hepatitis [2632] have been presented. The causes of hepatitis following arsenic therapy have been found to be homologous serum or B virus hepatitis in some instances and "cholangiolitic" hepatitis in others [785, 786, 3236]. The recognition of anicteric hepatitis [125, 2189] and the morphologic similarity between infectious hepatitis and infectious mononucleosis [148, 3404, 3452] have been based on liver biopsy examinations. Chronic hepatitis and the posthepatitic state have been described [125, 154, 215, 996, 1576, 1677, 1845, 2660, 2711, 3045]. A nonspecific hepatitis with mild diffuse hepatocellular degeneration, focal necrosis, and mesenchymal reaction occurs in

many infectious diseases, such as tuberculosis [2907]. In ulcerative colitis [1798] and peptic ulcer [2244] similar changes have been observed.

Liver Biopsy in Cirrhosis. The study of cirrhosis has been facilitated by liver biopsy. The alterations often simulate those in acute hepatitis, except that the lobular parenchyma is dissected by connective tissue septums. Criteria for the recognition of cirrhosis have been described [2763], and the pathologic, clinical, and laboratory findings have been correlated [3467]. The importance of biopsy in the differential diagnosis of cirrhosis with jaundice has been reviewed [775], and the pathology of alcoholic [431, 744, 1845, 2881] and nutritional cirrhosis [1491] has been described. Liver biopsy is important in recognizing the transition of viral hepatitis into cirrhosis [996, 1857, 1880, 2802].

Liver Biopsy in the Differential Diagnosis of Jaundice. The value of liver biopsy in the differential diagnosis of jaundice is still not fully established, despite many attempts to describe the morphologic changes in extrahepatic cholestasis [2802], or the histologic criteria for the differentiation of primary parenchymal damage from extrahepatic biliary obstruction [1390, 2365, 2632, 3549]. Intrahepatic cholestasis [786, 2758, 3510] and familial nonhemolytic jaundice [1857] have also been studied. The use of liver biopsy in the differential diagnosis of jaundice should be based on the understanding of the problem of differentiating those lesions in which hepatocellular degeneration is the primary factor from other lesions in which extrahepatic or intrahepatic cholestasis predominates. This differentiation is usually not difficult in earlier stages of jaundice, when secondary hepatocellular degeneration has not yet complicated extrahepatic or intrahepatic cholestasis. The differentiation in the biopsy specimen of intrahepatic cholestasis, or "cholangiolitis," from extrahepatic obstruction is far more difficult. During the first 3 weeks of jaundice the lesions can not be separated by biopsy. Since extrahepatic cholestasis involves the septal bile ducts and cholangiolitis does not, changes as a result of increased pressure in the bile ducts in the portal tracts indicate extrahepatic biliary obstruction. The absence of such changes 4 weeks after the onset of jaundice speaks against extrahepatic cholestasis. The efficacy of liver biopsy examination in the differential diagnosis of jaundice is illustrated by a series of 1,164 jaundiced patients; in 46, the biopsy was necessary after all other methods failed [289]; in

only four of these was the biopsy diagnosis incorrect.

Depositions in the Liver Recognized in Biopsy Specimens. Biopsy examination is especially valuable for the recognition of fatty metamorphosis of the liver, since biochemical hepatic tests do not clearly reflect such changes. Various types of fatty liver have been demonstrated [277, 3386], produced by protein malnutrition [739, 1172, 3499], alcoholism [431, 2993], obesity [3700], diabetes [1856, 3722], and after aureomycin therapy [974].

The deposition of other material such as iron in hemochromatosis [1602] or amyloid can be recognized.

Focal Lesions Found by Biopsy Examination. Secondary [359, 1552] and primary carcinomas [628] have been found. Miliary tubercles indicating hematogenous dissemination of tuberculosis have been demonstrated [676, 1632, 2213, 2851]. Liver biopsy is an efficient diagnostic method for the recognition of the follicles of sarcoidosis [1794, 2901, 3404], equal to or even more reliable than lymph node biopsy. Granulomas from brucellosis [1794, 3158], actinomycosis [1794], erythema nodosum [1794], histoplasmosis [581], and syphilis [3691] have been shown. Recognition of the etiology of the granuloma is often difficult, but establishing the presence of granulomatous disease by liver biopsy often suffices; the exact nature of the granuloma can be determined by other procedures.

Liver Biopsy and the Results of Therapy. Liver biopsy is a valuable tool in the recognition of the effect of therapy upon fatty metamorphosis [188, 1075, 1172, 1734, 2658, 3441]. It has been used for control of cirrhosis therapy [1075, 2698, 2758, 3441], but variations of the cirrhotic changes throughout the liver may result in erroneous conclusions, because the specimen is not representative. The effect of therapy with vitamin B preparations [1599] and sulfonamides [2686] has been studied. Biopsy examination has been recommended prior to shunt surgery [2921] or splenectomy [3591].

Liver Biopsy in Children. Needle biopsies have been performed in children, some less than one year old [1172, 1713, 2268]. Because of lack of cooperation on the part of small children, surgical biopsy is preferred by some investigators [3691].

Correlation of Biopsy and Biochemical Findings. The findings in the biopsy specimen have often been compared with the results of other hepatic tests [234, 581, 1771, 2372, 2662, 2946].

3040, 3554]. Most investigators emphasize the poor correlation which exists between the morphologic and the functional findings. Some correlation was demonstrated when individual histologic features were compared with the biochemical findings. Hepatic-cell damage was well correlated with the results of tests related to protein metabolism in liver disease in general [1074, 2645] and in cirrhosis [2651], hepatitis [3040], and cholelithiasis [2244].

Academic Information Obtained by Liver Biopsy. Liver biopsy specimens have been used to study the histologic distribution of alkaline phos-

phatase [596, 3045] and of vitamin A [1172, 2625]. Cultures of biopsy tissue have been made in many diseases [1151, 2898], including tuberculosis [2851].

As a result of biopsy studies, new information about classification of liver disease was obtained. The changes in passive congestion [3040, 3589] and in pregnancy [1588] were described. Biopsies have also been used in physiologic and chemical studies of carbohydrate metabolism [1489] and for chemical determinations of enzymes [709, 3045, 3499], fat [277], vitamin A [3638], nucleic acids, glycogen, and protein [3020] in liver tissue.

PART IV

Diffuse Diseases of the Liver

CLASSIFICATION AND NOMENCLATURE OF HEPATIC DISEASES

Liver diseases can be subdivided in several ways from structural and functional viewpoints. Diffuse diseases involving the entire liver, or at least a large continuous portion, are separated from focal diseases involving circumscribed areas. In diffuse diseases, impairment of hepatic function is noted, whereas in the focal diseases it is not necessarily present, since more than one-fourth of the entire liver, which suffices for most functions, is seldom involved [2202]. Disturbances of the bile flow also produce clinical manifestations, but only if the ducts of at least one entire lobe are compromised. Transitions between diffuse and focal lesions occur. For example, small granulomas may involve so much of the lobular parenchyma that they can be considered a diffuse lesion. The focal lesion is often complicated by diffuse hepatocellular involvement, which may be morphologically less impressive but functionally more significant than the focal lesion itself.

Any classification of hepatic diseases must include two types of conditions:

1. Hepatic diseases in which the hepatic involvement dominates the clinical picture, even though it may be secondary to other diseases; these diseases require special diagnostic and therapeutic considerations for the hepatic involvement itself.

2. Hepatic responses to disease processes which are systemic or are predominantly in another organ; these require little, if any, therapeutic or diagnostic consideration of the hepatic involvement.

These two categories often overlap considerably; therefore the following discussion includes both hepatic diseases and hepatic changes in other diseases.

The names of hepatic diseases in common usage vary not only in connotation but even more in

viewpoint. The attempt is made here to use pathologic-anatomic terms, qualifying them whenever possible by an etiologic designation. Such basic pathologic-anatomic names are useful not only for systematic cataloguing but also as diagnoses for liver biopsy specimens. Descriptive titles are used only when similar clinical, functional, and pathological entities are caused by different etiologic factors. In such instances the chapter is a review of previously listed stages of etiologic entities.

The problem of nomenclature is especially difficult in diffuse hepatic diseases. The attempt to use etiologic designations is hindered by the fact that the response of the liver tends to vary less with the etiologic agent than with the severity of the injury and the effect of concomitant factors, such as changes in circulation and nutrition [1497]. The selection of proper anatomically descriptive terms causes even greater difficulty. Rather than coin new terms or select terms accepted in foreign literature [3561], terms in common usage in the American literature were used to afford easy understanding. This entails a sacrifice of taxonomy for the sake of clarity. We refrained from using the broad term "hepatopathy" which would be appropriate for the major chapter headings and used the less systematic term "hepatic injury."

HEPATITIS VS. HEPATOSIS. The problem of nomenclature has been further complicated by the adoption of the term "hepatitis" for diseases in which the degenerative changes of the hepatic cells are structurally as well as functionally far more significant than the accompanying alterations of the vascular and mesenchymal framework. Although inflammation generally implies predominant alteration of the vascular and mesenchymal

systems, the term “hepatitis,” first used by Bianchi in 1710, has been applied to almost all diffuse liver diseases. Even where the inflammatory lesions are conspicuous, as in viral hepatitis, the degenerative changes of the hepatic cells are functionally more significant. Nevertheless, the term “hepatitis” is used for all conditions in which mesenchymal reaction is present. Designation of the lesion produced by extrahepatic biliary obstruction as biliary hepatitis is probably the most objectionable point, since the inflammatory component is not necessarily conspicuous. The value of this term lies in the fact that it permits the grouping of conditions in the differential diagnosis of which clinical acumen, laboratory tests, and liver biopsy are all

necessary. In this broad sense, the term “hepatitis” as used in American literature, is most practical. The use of the term “hepatosis” would seem rational to designate mainly degenerative conditions analogous to nephrosis or myocardosis, as suggested by Rössle [2797] and widely used in Germany [3070]. In order to make reading easier, the term has not been used, and “necrosis” or “hepatitis” was substituted, with apologies to the medical semanticists (Table 46).

Since several diseases can be listed under different types of hepatic injury, and since identical etiologic entities can produce either focal or diffuse lesions, cross references introduce each chapter of the various forms of hepatic injury.

Table 46 Names and Synonyms of Common Diffuse Diseases of the Liver

<i>Name</i>	<i>Synonyms</i>
Diffuse diseases of the liver.....	Diffuse hepatopathy
Toxic hepatic injury:	
Nonspecific reactive hepatitis.....	Infiltrative hepatitis, focal necrosis, benign lymphomas, “typhoid” nodules
Toxic hepatic necrosis.....	Toxic hepatitis or hepatosis, central toxic necrosis, toxic or chemical hepatopathy
Massive hepatic necrosis.....	Acute or subacute yellow or red atrophy or dystrophy
Postnecrotic cirrhosis (rare).....	See under Viral hepatitis
Viral hepatitis:	
Spotty necrotic form.....	Catarrhal jaundice, infectious hepatitis
Massive necrotic form.....	Acute or subacute yellow or red atrophy or dystrophy, fulminant or malignant hepatitis
Chronic nonfatal hepatitis.....	Posthepatic syndrome
Postnecrotic cirrhosis.....	Toxic cirrhosis, coarse nodular cirrhosis, postcollapse cirrhosis, posthepatic cirrhosis, multiple nodular hyperplasia
Cholangiolitis:	
Acute cholangiolitis.....	Pericholangiolitis, intrahepatic biliary obstruction, primary cholestasis
Cholangiolitic cirrhosis.....	Primary biliary cirrhosis, xanthomatous biliary cirrhosis, hypertrophic cirrhosis of Hanot
Hepatic injury from biliary obstruction:	
Biliary hepatitis.....	Obstructive hepatitis, extrahepatic biliary obstruction, obstructive jaundice, extrahepatic cholestasis
Biliary fibrosis (or cirrhosis).....	Cholestatic cirrhosis, obstructive cirrhosis
Infected biliary hepatitis.....	Intrahepatic cholangitis, cholangiohepatitis, purulent hepatitis
Secondary biliary cirrhosis.....	Cholangitic cirrhosis, cholangitic biliary cirrhosis, infected obstructive cirrhosis, infectious cirrhosis
Nutritional hepatic injury:	
Fatty liver.....	Trophopathic hepatopathy
Fatty liver with acute hepatic failure.....	Steatosis, fatty infiltration or degeneration
Florid cirrhosis.....	Subacute cirrhosis
Nutritional cirrhosis.....	Chronic toxic hepatitis, subacute portal cirrhosis, progressive alcoholic cirrhosis
Hepatic injury from congestion:	
Congestive fibrosis.....	Fatty, Laennec’s, atrophic, portal, or alcoholic cirrhosis
Congestive cirrhosis.....	Cardiac cirrhosis
	Cardiac cirrhosis

The liver is a target for many injurious substances which reach it via the systemic circulation or directly from the intestine in high concentrations. The susceptibility of the liver to damage results from its location and also from its detoxifying function, which can be overtaxed. The injurious substances are rarely toxins that, in the immunologic sense, are capable of eliciting antitoxin formation. In many instances, the noxious agent is not known, but its presence is surmised from the type of lesion found. Nevertheless, the term "toxic" is loosely applied to the entire group.

Many morphologic or etiologic entities can be discussed under toxic hepatic injury, although other pathogenetic factors indicate that they belong in other chapters. Diseases in which the hepatic lesion is not of sufficient significance have been mentioned under hepatobiliary syndromes. The hepatic diseases, in capital letters in the following listing, are described in more detail here, after an enumeration of etiologic factors producing toxic hepatic injury in experimental animals, where the role of the poison is established.

Toxic hepatic injuries can be divided from a morphologic standpoint into those with necrosis and those without necrosis. Necrosis varies in amount and location, although most toxic injuries affect the central zone of the lobule. Coagulation necrosis, with or without fatty changes, precedes breakdown of the cells and formation of anuclear fragments. The inflammatory response to these relatively large breakdown products is predominantly leukocytic and, less commonly, mononuclear. The entire liver is usually uniformly involved, and, in contrast to the condition in viral hepatitis, little difference is seen between right and left lobes.

Classification of Toxic Hepatic Injury

- I. Toxic hepatic injury without necrosis
 - A. Cloudy swelling (see Cloudy Swelling, under Hepatocellular Degeneration, Chap. 22)
 - B. Toxic fatty liver, usually the result of deficiency, although in a few instances, e.g., obstetrical hepatic failure [2404], it seems to be purely on a toxic basis
 - C. Perilymphangitis (see Perilymphangitis, under Focal Necrosis, Chap. 25)
 - D. "ALLERGIC" CHOLANGIOLITIS
 1. Arsphenamine
 2. Methyltestosterone
 3. Chlorpromazine
 4. Others
- II. Toxic hepatic injury with necrosis
 - A. NONSPECIFIC REACTIVE HEPATITIS; portal inflammation, intralobular inflammation
 - B. Mild central necrosis (see Central Necrosis, under Necrosis, Chap. 22)
 - C. TOXIC HEPATIC NECROSIS; extensive zonal or massive necrosis
 - D. Hepatic necrosis in eclampsia (see Hepatic Necrosis in Eclampsia, Chap. 49)
 - E. Hepatic congestion in thyrotoxicosis (see Hepatic Congestion in Thyrotoxicosis, Chap. 49)
 - F. Postoperative toxic hepatitis

EXPERIMENTAL HEPATIC INJURY

Toxic injuries have been produced in animal experiments in order to study the mechanism of the injury and to find protective and therapeutic agents.

The lesions discussed in the following chapter do not constitute a compendium of the almost unlimited number of ways of producing hepatic injuries, and reference is made to various reviews [833, 1735, 2503, 2797]. Examples of the dif-

Table 47 Substances Used in Various Experimental Animals to Produce Different Types of Hepatic Damage

Substance	Animal	Amount and route	Hepatic changes	Other organs affected	Reference
Alloxan	Rabbit	100-200 mg/kg intravenous—1 dose	Central necrosis		1938
Allyl formate	Rat	0.015 ml/100 gm intraperitoneal—1 dose	Peripheral necrosis and vascular degeneration		1937
Antibiotics: Aureomycin, chloramphenicol, Terramycin	Mouse and dog	75-100 mg/kg intravenous, intraperitoneal, subcutaneous, daily for 5 days	Fatty liver		1945
Arsphenamine	Dog	0.03-0.05 gm/kg intravenous, weekly for 1-5 weeks	Spotty or central necrosis, fatty liver on a high-fat diet		2271
BAL (2,3-dimercaptopropane)	Dog	15-30 mg/kg/day intramuscular for 4 days	Fatty metamorphosis		2139
Bromobenzene	Rat	0.05 ml/100 gm in corn oil—1 dose	Central necrosis		1822
Carbontetrachloride	Mouse	0.1 ml 40% CCl ₄ in olive oil—1 dose orally	Central necrosis		3240
		0.04 ml in olive oil orally, twice weekly for 2-39 weeks	Cirrhosis, tumors		3241
	Rat	10 ml in air or vapor, 10 min/day for 10-31 days	Cirrhosis, tumors		253
		0.05 ml/100 gm subcutaneous, twice weekly for 3-12 weeks	Fatty liver, cirrhosis		110
		0.2 ml/100 gm subcutaneous—1 dose	Hydropic degeneration, ischemia, central necrosis		1193
		0.033 ml/100 gm in mineral oil, intraperitoneal—1 dose	Fatty liver and central necrosis		1820
		0.5-1.0 ml orally—1 dose	Central necrosis		2108
	Cat	0.3 ml/kg subcutaneous—1 dose	Central necrosis and fat		481
	Dog	10-25 ml/week orally for 5-12 weeks	Cirrhosis		947
Carcinogens: p-dimethylaminoazobenzene	Rat	0.06% in diet for 4-5 months	Tumors with or without cirrhosis		2459
2-methyl or 3'-methyl-4-dimethylaminoazobenzene	Rat	0.064% in diet for 28 days	Increased mitochondria, bile duct proliferation (tumors on longer feeding)		3251
o-aminoozotoluene	Mouse	10 mg in glycerol per month for 10 months, subcutaneous	Cirrhosis, tumors	Lung and hemangioendotheliomas all over	72
2-acetylaminofluorine	Rat	3-9 mg/day in diet for 35 weeks	Cirrhosis, tumors	Multiple primary tumors	3006
Dibenzanthracene	Rat	3 mg/week in 3 doses, intraperitoneal, for 3-12 weeks	Fatty liver, decreased vitamin A		1198
	Rabbit	10 mg/week in 3 doses, intraperitoneal, for 2-7 weeks			
Chloroform	Rat	Anesthesia for 1 hr	Fatty metamorphosis, hepatic-cell degeneration, and necrosis		1213
		0.4-1.5 ml/kg in mineral oil, subcutaneous—1 dose	Extensive central necrosis		2128
	Rabbit	0.25 ml/kg in mineral oil, subcutaneous every other week for 1-8 months	Nodular cirrhosis	Kidney, lung	1000
	Dog	Anesthesia for 30 min	Hyaline central necrosis		2299
Cholestone	Rat	0.20-0.27 mg/100 gm, intraperitoneal, 21-60 days	Increased mitotic activity and fat		2914
Coramine	Rat	1% in diet for 4-5 weeks	Increase in liver weight and fat		387
DDT and analogues	Rat	0.02-0.16% in diet for 1 year	Coagulation necrosis, hyaline and fatty degeneration	Spleen and kidney	2035
Dextran	Mouse	1.0 ml 6% solution intravenous—1 dose	Masses in hepatic cells and blood vessels	Kidney	2070
2,6-diaminoanthrapyrimidine	Mouse	4 mg/20 gm twice a day for 1-3 days or twice a week for 1-2 weeks, orally	Blue staining of hepatic cell, central necrosis, giant-cell formation		1385
Dinitro-o-cresol	Rat	20 mg/kg subcutaneous—1 dose or daily for 1 week	Increased liver glycogen and decreased glycolysis		190
Ethionine	Rat	75 mg/100 gm intraperitoneal, in 3 doses, 2½ hr apart, in females	Fatty liver	Pancreas	1830
2,4,6-trichloride	Rat	0.2-0.5% in diet for 28-104 days	Fibrosis, tumors, cholangiofibrosis	Pancreas	2635
Mapharsen	Dog	1 ml/kg subcutaneous—1 dose	Fatty degeneration	Kidney	1889
		3.4-9.1 mg/kg intravenous—1 dose	Bromsulphalein retention, fatty liver, congestion, cloudy swelling	Kidney	1889

Substance	Animal	Amount and route	Hepatic changes	Other organs affected	References
Monocrotaline	Rat	46 mg/kg intraperitoneal every other day until death	Centrolobular necrosis	Kidney	2718
ectin	Mouse	0.5-2.0 ml intravenous—3 doses in 2 weeks	Focal necrosis	Kidney	2756
phosphorus	Rat	0.75 mg subcutaneous—2 doses	Fatty liver		833
propylene dichloride	Rat	2,200 ppm for 4-7 hours—1-5 times	Central necrosis and fatty degeneration	Kidney, adrenal	1487
pyridine	Guinea pig	0.1-1.0% in diet for 2-3 weeks	Extensive central necrosis followed by postnecrotic scarring	Kidney	186
Radioactive colloidal gold	Rat	10-40 microcuries/gm intravenous—1 dose	Central coagulation, necrosis, atrophy, cirrhosis		1834
Selenium	Rat	5-10 ppm in diet, 2-4 months	Subacute necrosis, congestion		758
		5-10 ppm in diet, 12-24 months	Cirrhosis, tumors		2004, 2422
Senecio		2.2 mg/kg subcutaneous, daily for 28 days	Fatty degeneration, focal necrosis		3002
Longilobine	Mouse	56-100 mg/kg intravenous—1 dose	Central necrosis, congestion, hemorrhage		1456
Retrorsine					
Silica (ground quartz)	Rat	0.010-0.015 mg/kg orally—1 dose	Hemorrhagic zonal necrosis		3007
	Dog	7.5-10 gm suspended in saline, into mesenteric vein in divided doses at monthly intervals	Chronic portal-vein obstruction		3439
Stilbamidine	Rabbit, mouse	10-100 mg/kg orally—1 dose	Hepatic necrosis, peripheral fat	Kidney	2979
		0.02-0.013 mg/kg/day, subcutaneous, 1-8 weeks			
Sulfaguanidine	Rat	50 mg/kg/day orally for 11 doses	Hepatic-cell degeneration and focal necrosis	Marrow, spleen, thyroid, heart	1296
		1% in diet for 5 months			2687
Thiourea (and thioacetamide)	Rat	0.4 gm in 10% solution, intraperitoneal, for 3 days	Sinusoidal congestion, hydropic swelling, increased mitosis	Thyroid	1028
		0.1% in diet for 2 years	Hyperplasia, hepatoma		
TNT	Rat	0.3% in diet for 60-200 days	Fatty liver		
	Rabbit	0.2 gm/kg subcutaneous, every other day for 60 days	Fatty liver		3099
	Guinea pig	0.2 gm/kg orally for 42 days	Central fat		
	Cat	0.02 gm/kg subcutaneous, daily for 17 days	Central fat		1172
Trypan blue	Rat	0.5-1.0 ml of 1% solution parenterally at weekly or fortnightly intervals for 1-64 weeks	Necrosis, fibrosis, reticulum hyperplasia, tumor formation		2159
Uranium nitrate	Dog	4 mg/kg subcutaneous—1 dose	Diffuse hepatic-cell degeneration and fatty metamorphosis		810
Urethane	Rat	1-5 ml of 10% solution intraperitoneal—1 dose	Congestion, hemorrhage, endothelial damage		
		0.5 ml of 10% solution intraperitoneal daily for 4-14 days	Edema, spotty necrosis, endothelial damage		3270
Xylidine	Dog	0.22 mg/liter air, 6 hr/day for 4-5 weeks	Fatty liver, central necrosis		

ferent types of experimental injury which can be reliably reproduced are listed. Methods that have been tried in the laboratory of the authors, chiefly on rats, are given preference in the description. Examples of the effects of various poisons in different animals and the routes of administration are listed in Table 47.

Central Necrosis with Fatty Metamorphosis. Central necrosis associated with fatty metamorphosis is produced by carbon tetrachloride or by other, often halogenated, substances with similar volatility and fat solubility. Substances such as carbon tetrachloride are found in the mitochon-

dria of hepatic cells during intoxication and are thought to interfere with intermediary metabolism in the Krebs tricarboxylic acid cycle. The common denominator, aside from toxicity, responsible for the necrosis seems to be disturbance of centrolobular circulation, as demonstrated by vital microscopic [3463] and injection [1193, 2175] techniques. The lesion has therefore been considered to be the result of anoxia from disturbed circulation [76]. The centrolobular necrosis disappears in a few days, but if the exposure is often repeated each time before necrosis disappears, cirrhosis formation results.

ACUTE CARBON TETRACHLORIDE INTOXICATION. The morphologic effects of carbon tetrachloride (CCl_4) intoxication have been studied extensively in rats [116, 467], mice [3240], cats [481], dogs, and rabbits. Except for minor species variations [833, 2337], the changes are similar in most animals. After intraperitoneal injection of as small a dose as 0.3 ml carbon tetrachloride per kilogram body weight, diluted tenfold in mineral oil, changes, which start within a few hours, have been produced consistently in rats. These are hydropic swelling of the centrilobular hepatic cells and obliteration of many sinusoids [1193, 3463]. After 24 hours, simultaneously with a grossly visible exaggeration of the lobular pattern, centrilobular necrosis develops, associated with hydropic swelling of cells lining the necrotic area and severe fatty changes in the intermediate zone which reach almost to the portal tracts [467, 1820] (Fig. 131A). This is associated with a central loss of basophilia but not necessarily with a decrease of the nucleic acid content chemically [477, 976], and an increase of basophilia on the periphery of the lobule [477], especially during anatomic repair [1524].

Chemical [3367] and histochemical [1524, 3240] alterations, as well as alteration of the hepatic function, during this stage have been studied [253, 1820, 3635]. The severity of the lesion can be measured histologically by the extent of the necrosis and fat accumulation and chemically by reduction of hepatic esterase activity, increase of hepatic lipids, hepatic and serum-alkaline phosphatase activity, serum bilirubin, serum xanthine oxidase, serum phospholipids, and Bromsulphalein retention [1820, 2637]. After 48 hours recovery starts, and within 1 or 2 weeks no residual changes are seen. The oral or intraperitoneal routes of administration which result in portal absorption cause the changes more consistently than the subcutaneous route [2108]. Direct injection into the portal vein rapidly produces massive hepatic necrosis, called "toxic infarction," which does not involve the entire liver and results in postnecrotic scarring [468].

A high-fat diet increases the toxicity. High-carbohydrate and -protein diets are protective. Choline is ineffective, and methionine protects or improves the liver changes only in animals on low-protein diets [477, 833, 1326], although short-term low-protein diets are not detrimental [477]. Vitamin E [116, 1553], vitamin B_{12} [1826], sulfhydryl compounds [426], and sulfonamides [1918]

are reportedly beneficial, while cortisone is not [116]. Regenerating liver is more resistant to carbon tetrachloride injury [950].

CHRONIC CARBON TETRACHLORIDE INTOXICATION. Chronic carbon tetrachloride injury is most consistently produced by inhalation in a special chamber. Biweekly intraperitoneal injections of 0.3 ml per kg [467, 2635] or subcutaneous injection of 1.0 ml per kg [110, 3389] also produce cirrhosis after several weeks. A diet containing 5.0 per cent carbon tetrachloride placed in the cages twice daily to avoid evaporation results in cirrhosis formation within 8 weeks [110, 2637] (Fig. 131D). In this stage, hepatic and serum-esterase activity are reduced, and hepatic and serum-alkaline phosphatase activity are elevated [2637]. Regeneration after hepatectomy is poor [2202]. If the injections are discontinued, recovery occurs, with a decrease of the collagen already formed [2351]. With continued exposure, portohepatic venous anastomoses [3389] and, in even later stages, hepatomas [891] develop.

CHLOROFORM. Chloroform produces essentially the same lesion as carbon tetrachloride. Most of the chloroform studies are relatively old and chiefly concern dogs and rabbits. The lesion has been produced mainly by inhalation anesthesia, usually from $\frac{1}{2}$ to 2 hours in duration. Cirrhosis formation has been observed after repeated exposure [1000]. Mid-zonal necrosis in hyperthyroid rabbits is an interesting modification of the intoxication, emphasizing the importance of accompanying factors [294], especially tissue nutrition [752, 2128]. The chloroform injury is aggravated by protein depletion and by increased lipid deposits in the liver. The protective role of adequate protein nutrition was first and best established in depleted animals [1213, 2299]. The effect of methionine is probably related to protein metabolism [2299]. Urethane synergistically increases the toxic effect of chloroform [2091].

PHOSPHORUS. In earlier studies phosphorus was used to produce severe fatty infiltration with necrosis in the lobular periphery. Cirrhosis formation has also been observed [2187].

LEAD. Rats on a high-fat diet intoxicated with lead acetate develop central necrosis with fatty metamorphosis, apparently because of removal of sulfhydryl groups during excretion of the metal [570].

OTHER COMPOUNDS. Ethylene dichloride produces central necrosis with fatty changes similar to those produced by chloroform. This is partly

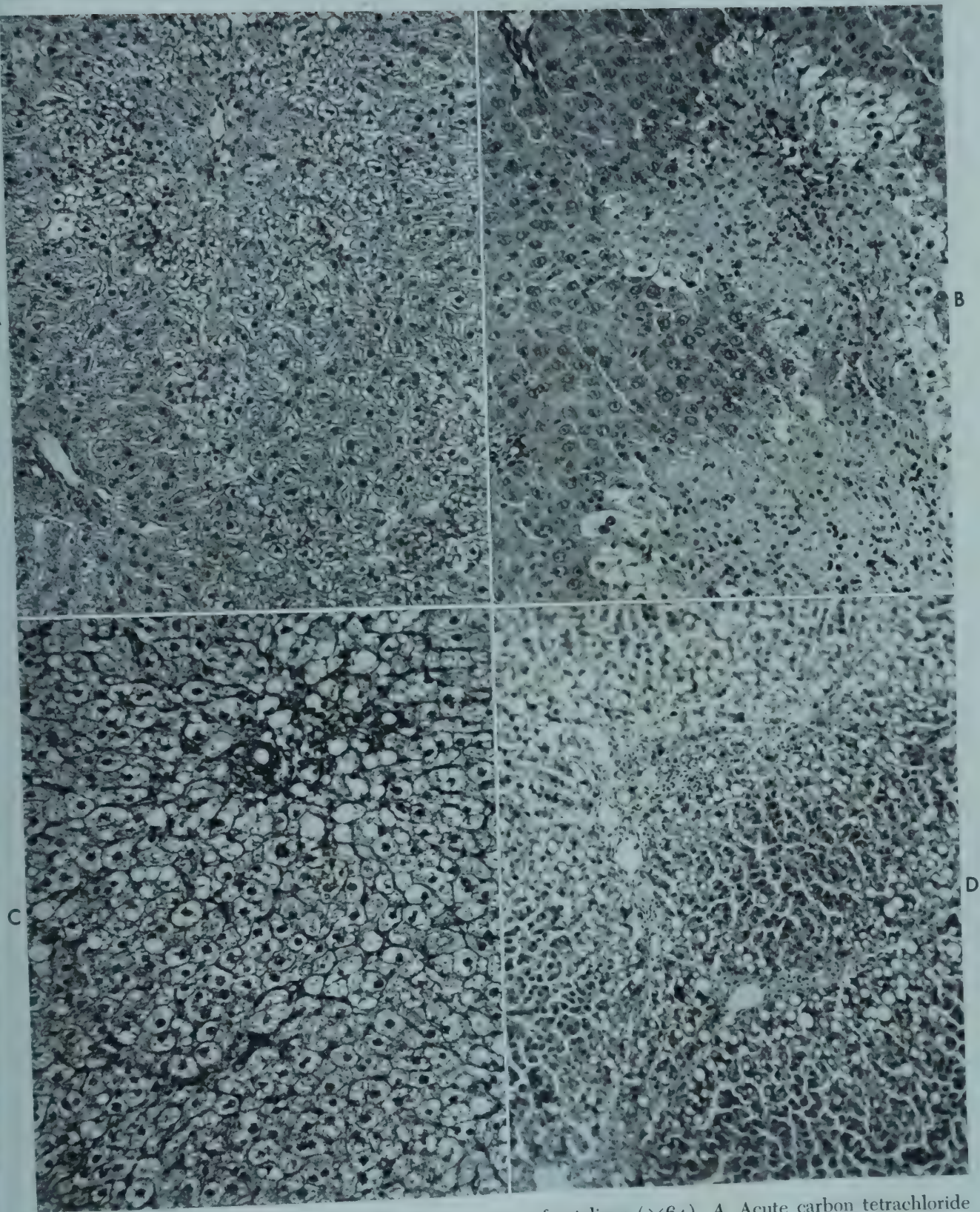


FIG. 131 Hematoxylin-eosin-stained paraffin sections of rat liver ($\times 64$). A. Acute carbon tetrachloride intoxication. Necrosis and disappearance of hepatic cells in the centrilobular zone. Hydropic swelling and fatty metamorphosis in the intermediate zone. B. Acute bromobenzene intoxication. Acidophilic and fatty metamorphosis in the intermediate zone. Hydropic degeneration of hepatic cells bordering the necrosis. C. Acute ethionine intoxication. Diffuse fatty metamorphosis without necrosis. D. Chronic carbon tetrachloride intoxication. Distortion of the lobular patterns by connective tissue septa connecting the central fields. Focal necrosis and fatty metamorphosis. (Popper, H., de la Hueriga, J., and Koch-Weser, D.: *J. Lab. & Clin. Med.* 39:725, 1952.)

prevented by methionine but is not significantly prevented by choline. Prevention by adequate protein nutrition is less clear-cut than with chloroform [833, 1488]. Trinitrotoluene (TNT) also produces a fatty liver followed by necrosis [1498].

Central Necrosis without Fat. Necrosis in the centrolobular zone is the most common response of the liver to hepatic injury. It has been produced by many substances, with varying degrees of regularity. In some instances, the lesion is the result of circulatory interference. Intraperitoneal administration of 0.05 ml bromobenzene in 0.25 ml olive oil per 100 gm body weight produces an extensive central necrosis with hydropic swelling on the periphery, central inflammatory reaction, and a moderate degree of acidophilic necrosis within 48 hours (Fig. 131B). Hepatic and serum alkaline phosphatase, serum bilirubin, and Bromsulphalein retention are elevated, and hepatic esterase is reduced [1823]. The lesion can be prevented by simultaneous administration of methionine or cysteine. Complete recovery occurs in 72 hours.

Central necrosis has been produced in various animals by organic arsenic compounds [833, 2503]. The lesion is not readily reproduced, although protein in the diet protects the liver [2271]. Tannic acid [3555], pyridine [186], chlorinated naphthalene, and bacterial products such as botulinus toxin cause central necrosis [3025]. Toxic alkaloids from senecio plants are responsible for extensive central changes in animals. These are thought to result from damage of the central vein, with associated necrosis [1456, 3007].

Peripheral Necrosis. Intraperitoneal administration of 0.015 ml allyl formate per 100 gm body weight diluted in 2 ml Ringer's solution produces severe peripheral necrosis within 24 hours in rats. This is supposedly reduced in extent by penicillin [894] and is associated with sinusoidal congestion and hemorrhages in the portal tracts [2625]. The lesion is probably caused by primary damage of the capillary wall [2832]. Peripheral necrosis is also produced by phenyldichloroarsine [758], stilbamidine [2979], and manganese [1735].

Focal Necrosis. Areas of focal necrosis can be produced in many ways, such as with toxic doses of beryllium sulfate [2975], but their erratic appearance makes them unsuitable for comparative studies.

Diffuse Hepatic Degeneration. Diffuse hepatic degeneration, not necessarily associated with necrosis, has been produced in various ways. Ura-

nium nitrate administration causes hepatocellular damage plus fatty infiltration [2159]. Diphtheria toxin also causes diffuse alterations [2624]. A reproducible diffuse degeneration results after 8 weeks on a synthetic diet containing 5 per cent bromobenzene fed twice daily to avoid evaporation. The hepatic-cell plates are distorted, and hydropic swelling and clumping of the cytoplasm are noted in the intermediate and peripheral zones in the absence of increased fat accumulation (Fig. 132B). Hepatic and serum-esterase activity are reduced, and hepatic and serum-alkaline phosphatase activity are increased [2637].

A different and more severe type of diffuse hepatocellular degeneration associated with necrosis of isolated hepatic cells is produced within 5 weeks by feeding a synthetic diet relatively poor in choline and riboflavin, containing not more than 0.4 per cent methionine in its protein, and supplemented by 0.5 per cent ethionine. The diffuse hepatocellular degeneration is associated with regeneration, excessive proliferation of ductular cells in single-file cords and clusters, interstitial infiltration with reticulum cells and lymphocytes, and an excess of reticulum fibers (Fig. 132A) (see Ethionine Administration, under Deficiency of Specific Amino Acids, Chap. 50). Bromsulphalein retention, hyperbilirubinemia, bilirubimuria, decrease of hepatic protein, lipids, and esterase activity, and increase in hepatic water content and alkaline phosphatase activity develop. In the serum, alkaline phosphatase activity is increased and esterase activity is reduced [2635]. Eventually this degeneration progresses to post-necrotic cirrhosis and to tumor formation. A similar lesion can be produced by feeding thioacetamide [1028].

Other Hepatic Changes Experimentally Produced by Poisons. **ENDOTHELIAL DAMAGE.** Toxic damage to the sinusoids is associated with many lesions but seems to be most important in injuries produced by allyl formate [2832], urethane [810], and trypan blue [1172].

ABNORMAL REGENERATION. Almost every type of hepatic injury is associated with active and occasionally bizarre regeneration. Giant-cell regeneration is conspicuous after treatment with radio-active colloidal gold [1834]. Excessive mitoses without liver damage have been produced by thiourea [2687] and colchicine [2314]. Suppression of mitosis results from treatment with nitrogen mustard [1904]. Toluylenediamine, which has been used to produce experimental jaundice,

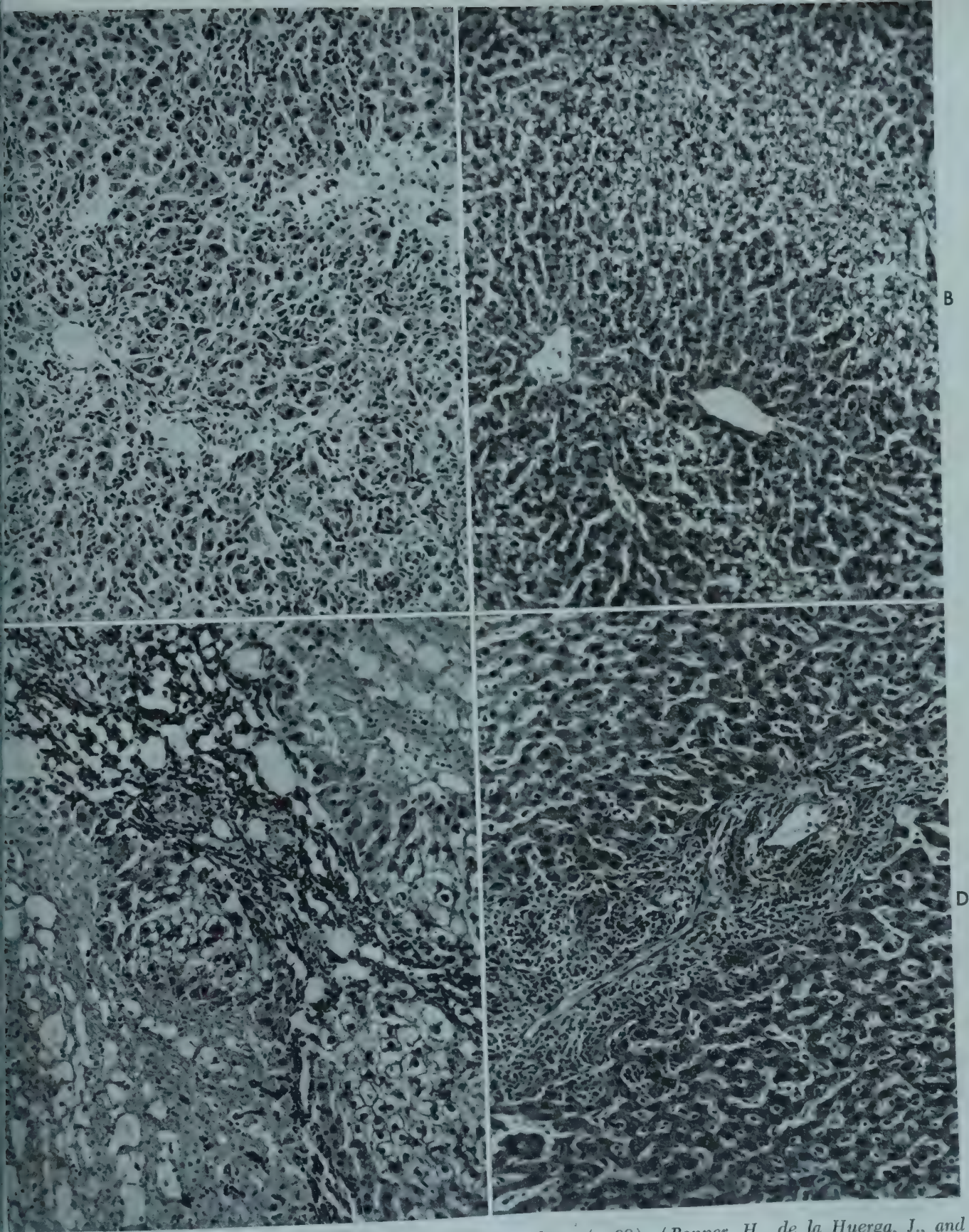


FIG. 132 Hematoxylin-eosin-stained paraffin sections of rat liver ($\times 68$). (Popper, H., de la Hueraga, J., and Koch-Weser, D.: *J.Lab.&Clin.Med.* 39:725, 1952.) A. Chronic ethionine intoxication. Diffuse degeneration, single-cell necrosis, and regeneration of hepatic cells are associated with excessive proliferation of ductular cells in single file cords and clusters, infiltration by reticulum cells, and excess of reticulum fibers, all obscuring but not distorting the lobular architecture. B. Chronic bromobenzene intoxication. Hydropic degeneration and clumping of cytoplasm of hepatic cells chiefly in the peripheral zone, where the hepatic-cell plates are irregularly arranged. C. Chronic butter-yellow intoxication. Necrobiosis, necrosis, and irregular regeneration of hepatic cells, some of them arranged in nodules. Irregular proliferation of ductules. D. Common-duct ligation. Proliferation of bile duct and ductules and cellular infiltration in portal tracts. Bile stasis, Kupffer cell mobilization, and slight hepatic-cell damage.

causes ductular proliferation in addition to hemolysis and hepatic-cell damage [943].

FATTY LIVER WITHOUT NECROSIS. Diffuse fatty metamorphosis of the liver without necrosis is usually produced by nutritional imbalance. The acute fatty liver produced by intraperitoneal administration of 100 mg ethionine per 100 gm body weight to female rats is an intermediary between toxic and nutritional fatty liver (Fig. 131C) (see Ethionine Administration, under Nutritional Deficiencies, Chap. 50). This fat accumulation is associated with increased Bromsulphalein retention but not with enzyme changes [1820]. Acute fatty metamorphosis can also be produced by ethionine administration to dogs.

CIRRHOSIS FORMATION. Various types of cirrhosis have been produced experimentally by toxic substances, often with eventual progression to carcinoma formation. These substances include selenium [758, 2422], carbon tetrachloride [655, 891], allyl formate [945], pyridine [186], butter yellow [2489], thiourea, thioacetamide, ethionine [2635], and other carcinogenic substances (see earlier in this chapter).

ETIOLOGIC FACTORS IN HUMAN TOXIC INJURY

The examples of toxic injury to the liver in man are so numerous that reference is once more made to reviews [833, 1735, 2503, 2579]. The histologic alterations caused by a drug vary from patient to patient, and they are determined less by the drug than by factors inherent in the liver itself or by contributing factors [3367]. Also, variations in the clinical course are not related to the offending substance, and therefore the pathologic and clinical manifestations as well as the laboratory findings are discussed without special reference to the etiologic agent.

Toxic injury is caused by (1) exogenous factors such as accidental ingestion or administration for suicidal or homicidal purposes; (2) exposure to industrial poisons and other occupational hazards; (3) intake of medicinals as part of therapy; (4) endogenous factors. Phosphorus, chloroform, and mushrooms were formerly common causes of toxic hepatic injury, but now poisonings resulting from medicaments are more frequently encountered.

Endogenous Factors

BACTERIAL TOXINS. Bacterial toxins developed in the body of the host, rather than bacteria them-

selves, are responsible for the liver damage which occurs in diphtheria, gas bacillus infection, and many other conditions. Nonspecific hepatic injuries demonstrable by structural and functional alterations in many infections, such as pneumonia [3723] and tuberculosis [2907, 2992], probably belong in this group. It is difficult to differentiate the effect of bacterial toxins from the results of stress, shock, and anoxia produced by the bacterial infection. Similarly, in many examples of endogenous toxins, the secondary effects on the liver or damage to other organs may be more important than the direct effect of the usually mysterious toxic substance upon the liver.

INTESTINAL TOXINS. The early concept of intestinal toxemia, or autointoxication, was formerly discredited, but the beneficial effect of antibiotic treatment in experimental hepatic necrosis and cirrhosis has raised new interest in this problem (see Dietary Necrosis, Chap. 50).

TISSUE-BREAKDOWN PRODUCTS. The evidence for tissue-breakdown products damaging the liver is still circumstantial. The hepatic injury in ulcerative colitis [1753, 1798, 2618], gallbladder diseases without involvement of the remainder of the biliary tract [2244, 2279], and pancreatitis [2884] is probably mainly the result of tissue-breakdown products. Liver damage is present in patients with carcinomas anywhere in the body, especially in the stomach [7] or colon [152], possibly on the same basis, although absorption of injurious substances from the intestinal lumen can not be excluded. The liver damage is more severe with intrahepatic metastases, which explains the jaundice often seen with metastatic carcinoma in the absence of obstruction of the major bile ducts, or in lymphomas, particularly if hepatic infiltration is present. The loss of liver tissue by carcinomatous or lymphomatous infiltration or intrahepatic obstruction alone is not sufficient to explain the findings (see Metastatic Tumors in the Liver, Chap. 59).

Hepatic injury following tissue breakdown, particularly after infarcts [3470], is possibly the result of an unknown toxin formed by the necrotic tissue. Burns produce central hepatic necrosis 2 to 5 days after the injury [437, 3627] because of shock, anoxia, hemoconcentration, and cardiac failure, in addition to possible toxic factors from tissue breakdown [362]. The appearance of the lesion is not uniform, and some authors associate the hepatic changes after burns with therapy, such as administration of tannic acid [143]. Some of the hepatic

lesions in burns simulate those found in yellow fever [210].

BLOOD TRANSFUSIONS. Whether blood transfusions, not strictly an endogenous factor, damage the liver is not established. Some instances of such damage in the earlier literature probably were actually serum hepatitis. Liver damage with jaundice a few days after blood transfusions is possibly the result of a combination of the following factors: (1) excretion by the hepatic cells of excessive serum bilirubin resulting from hemolysis of part of the transfused erythrocytes, even in the absence of transfusion reactions, since about 30 per cent of the infused red cells are rapidly destroyed; (2) breakdown products of heme potentially injurious to the hepatic cells [945]; (3) breakdown products of globin, especially peptones and peptides, which irritate the hepatic cells; (4) blockade of the Kupffer cells by hemoglobin-breakdown products. The hepatotoxic effects of blood transfusions with or without preceding liver damage require further elucidation.

HORMONAL FACTORS. Hormones and substances with hormonal activity may be responsible directly or indirectly for hepatic injury. These include thyroid hormone (see Hepatic Congestion in Thyrotoxicosis, Chap. 49) and desoxycorticosterone [3080]. In pregnancy and eclampsia, hepatic injury frequently occurs, although the toxin is not known (see Hepatic Necrosis in Eclampsia, Chap. 49). Stilbesterol and diethylstilbesterol are said to be hepatotoxic [913, 3308], but convincing histologic or functional evidence has not been found [1329], and under some circumstances they are lipotropic [1324]. The alteration resulting from methyltestosterone [397, 3559, 3654] is discussed later (see Methyltestosterone Jaundice, later in this chapter).

Exogenous Factors

Hepatic injuries resulting from exogenous factors occur (1) from substances absolutely toxic which produce hepatic injury regularly after there has been exposure to toxic amounts; (2) from substances relatively toxic, in that hepatic injury develops only in the presence of contributing factors, such as infectious diseases or malnutrition; (3) as hepatic side reactions after exposure to substances primarily damaging to other organs, the liver damage being the result of shock, anoxia, or destruction of the other organ; (4) from idiosyncrasy in a few persons who react to drugs in doses innocuous for the large majority of persons; (5)

as instances of viral hepatitis incorrectly ascribed to injurious substances; (6) from substances, including alcohol, that alter the nutritional status.

Absolute Toxicity. CARBON TETRACHLORIDE. One of the common poisons causing hepatic injury by ingestion or inhalation is carbon tetrachloride (CCl_4) used in dry cleaning, in fire extinguishers, as a solvent, and as an antihelminthic. In recent years the number of instances of hepatic injury tentatively or definitely attributed to carbon tetrachloride has been rapidly increasing [2473]. Fatalities from both acute and, less frequently, chronic intoxication occur, especially in alcoholic persons, since alcoholism severely aggravates the lesion [2336]. Following exposure, renal injury often dominates the clinical course, with the resulting toxic nephrosis causing death. The hepatic lesion is usually inversely as severe as the renal lesion.

Clinical Manifestations. In mild instances of acute exposure, irritation of the eyes, headache, nausea, and dizziness appear. After more severe exposure, for instance as a result of inhalation of fumes, mental confusion, vomiting, colicky abdominal pain, and hemorrhagic tendencies develop rapidly. Renal manifestations, culminating in azotemic oliguria, start between 1 and 4 days after exposure and may persist for 2 weeks. Manifestations of toxic hepatic necrosis associated with jaundice usually develop a few days after exposure. Following oral ingestion, the same manifestations develop, with gastrointestinal symptoms, including a burning sensation in the mouth and bloody vomitus and diarrhea. After both oral ingestion and inhalation, death may result from respiratory or cardiac failure within the first day or two, or from renal or hepatic failure afterward. Chronic exposure, usually as a result of repeated inhalation, produces a less characteristic picture of fatigue, anxiety, headaches, peripheral neuritis, gastrointestinal symptoms, hyperlipemia, and jaundice. Methionine is supposedly beneficial in treatment, especially of the acute form [198, 879].

Structural Alterations. Fatty degeneration of the liver and centrilobular necrosis, followed by inflammatory reaction, collapse of the central zone, and early regeneration starting from the periphery of the lobule, are seen [2336, 2554] (Fig. 136A). The hepatic lesion is usually most severe in patients dying within the first 4 days after a single exposure; it regresses at the time when the most severe renal lesions are noted. The renal lesions are characterized by toxic necrotizing nephrosis of the proximal convoluted tubules with segmental

acute nephrosis (lower nephron nephrosis) and biliary nephrosis in addition. The pulmonary changes that are frequently seen are the result of uremia. In rare instances of chronic intoxication, postnecrotic cirrhosis develops.

OTHER ORGANIC SOLVENTS. Many halogenated hydrocarbons [2473], such as ethylene dichloride [2040], tetrachlorethane [1309], and methyl chloride [3655], are similar solvents producing hepatic injury and jaundice. Ethylene glycol produces centrilobular hydropic degeneration, but the renal changes are more important and may cause death [3097]. Chlorinated naphthalene produces diffuse or mid-zonal necrosis [3246], while naphthalene alone in moth balls causes hemolytic jaundice [3732]. DDT produces a hepatic lesion which is said to be characteristic for the chlorinated hydrocarbon group of insecticides; namely, hypertrophy and increased cytoplasmic acidophilia of the centrilobular hepatic cells and margination of cytoplasmic granules, with hyalinization of the rest of the cytoplasm [1028].

INORGANIC POISONS. Phosphorus is a hepatotoxic agent which was often ingested during the last century as an abortifacient, in suicide attempts, and accidentally in matches, firecrackers, or rat poison, especially by children. Occasional accidental ingestion in recent years has resulted in death [782, 1896, 2850], often without jaundice, within 24 hours [1470]. Those who survive the immediate effects become jaundiced after several days. The liver at this time usually shows severe fatty metamorphosis with focal or peripheral necrosis and portal inflammation in biopsy specimens. Sometimes fat is absent. Hemorrhagic tendencies and central nervous system manifestations occur [943]. In later stages, portal fibrosis has been observed [1896].

PLANT POISONS. The best-known plant poison that produces hepatic damage is amanita toxin from the poisonous mushroom, *Amanita phalloides*. It causes an almost choleralike gastroenteritis, lasting 1 to 2 days, usually followed, after 5 days, by severe jaundice, tender hepatomegaly, bilirubinuria and urobilinogenuria, hypoglycemia, and central nervous system manifestations [943, 1298, 1470]. Coma, hemorrhagic tendencies, and death supervene within 10 days. Those who survive the acute phase either gradually recover after a stormy period of severe jaundice or develop cirrhosis with all its sequelae. In the acute stage severe fatty infiltration, necrosis, and cholestasis are present in the liver [1801]. In addition fatty

and degenerative changes are noted in the kidney, myocardium, and the central nervous system [1298].

In South Africa, instances of human senecio poisoning have been reported [3005]. This weed, which is also responsible for liver damage in sheep, cattle, and horses in the United States [1456], leads to clinical manifestations when it is a contaminant of flour. The signs and symptoms are a rapid onset of ascites, hepatomegaly, nausea, and vomiting, with obstruction of the central and sublobular hepatic veins and subsequent centrilobular necrosis with central and peripheral fibrosis [3005, 3007]. In Jamaica, bush teas which contain senecio alkaloids are consumed and have been accused of causing hepatic injury in malnourished children (see Infantile Sclerosis, under Tropical Malnutrition, Chap. 51).

Relative Toxicity. Many substances are toxic only in some persons, while other persons, similarly exposed, are spared. In some instances, nutritional deficiency, disease, or other contributing factors are responsible for the increased susceptibility, although differentiation from idiosyncrasy is not always clear.

METALS. Treatment with arsenicals, especially arsenates, arsenites, arsphenamine, neoarsphenamine, and Mapharsen, is followed by hepatic injury and jaundice in some individuals. This type of injury occurred more frequently during and following antisiphilic treatment with arsenicals [2573], varying in incidence with the preparation used [3130]. Originally, therapy of the syphilitic infection was held responsible, but syphilitic hepatitis with jaundice is very rare in adults (see Hepatic Syphilis, Chap. 54). In most instances, the antisiphilic treatment was responsible, causing one of three hepatic conditions.

1. Primary toxic effect of arsenicals. This is present when the jaundice appears early and in combination with other toxic manifestations, including skin eruptions, such as erythema of the ninth day. It has been considered a Herxheimer reaction and is actually a hepatic side reaction. It has been experimentally produced, with the histologic appearance of the liver similar to that seen in fatal human hepatic necrosis following arsenotherapy [2632].

2. "Allergic" cholangiolitis [1377] (see Allergic Cholangiolitis, later in this chapter).

3. Serum hepatitis. Hepatitis which develops weeks or months after the beginning of antisiphilic treatment may be "syringe-transmitted" hepa-

itis, which is viral hepatitis clinically and pathologically [785, 2609, 2801]. In the past, this has been the largest group (see Syringe or Instrument-transmitted Hepatitis, under Epidemiology, Chap. 42). Whether the arsenicals predispose to viral infections is questionable [2315].

Oral administration of arsenic in Fowler's solution reportedly leads to cirrhosis [1072].

Bismuth therapy has also been associated with jaundice. Most instances are viral hepatitis, but a few seem to be caused by bismuth itself [1687].

Gold therapy has rarely caused icteric or anicteric hepatitis [90, 515]. Death from ulcerative colitis and hepatic necrosis occurred in one instance [69].

Industrial chromium intoxication causes acute toxic hepatitis [2528]. Manganese produces brain changes with hepatic injuries [968]. Antimony compounds produce jaundice in some instances. In lead poisoning, nuclear inclusion bodies are found in the hepatic cells [3445] which occasionally aid in establishing the diagnosis.

ANESTHETICS. Chloroform anesthesia increases serum bilirubin in most persons within 24 hours, and jaundice develops in some persons within 1 or 2 days. Exceptionally, clinical evidence of hepatic failure appears associated with convulsions and with subsequent shrinkage of the liver. At autopsy centrilobular necrosis and hemorrhage with severe fatty degeneration in the intermediate zone and cholestasis are found [1470]. The type of injury depends on the preceding condition of the patient, especially in obstetrical practice, where delayed chloroform poisoning was previously common [3033]. With discontinued use of chloroform as an anesthetic, this lesion has become rare. Hepatic injury also occurs after accidental or intentional ingestion of chloroform.

Other anesthetics, including ethyl chloride and Avertin, produce hepatic injury only in exceptional instances. Hepatic function is slightly impaired as a result of any general anesthesia, not necessarily related to its duration as recognized by hepatic tests [970]. Barbiturates are not hepatotoxic [3011].

In patients with liver disease the choice of anesthetics is difficult because of the increased susceptibility of the liver to anoxia, the reduced ability of the liver to detoxify some agents such as barbiturates, and the hepatotoxic action of some anesthetics. Those producing anoxia, such as nitrous oxide, and those that are toxic, such as chloroform, should be avoided. Spinal anesthesia

or local procaine block is preferable; next in order of preference are cyclopropane and ether. Intravenous barbiturates should be used only as a last resort.

OTHER ORGANIC COMPOUNDS. Tannic acid applied to patients with burns produces central necrosis which is not the result of the burn alone [3555] (see Central Necrosis, under Toxic Hepatic Necrosis, later in this chapter). Pyridine used as an anticonvulsant has been reported to cause liver damage [2621]. DDT ingestion has resulted in death with central necrosis [1996, 3101]. Prolonged urethane administration sometimes leads to severe liver damage [2253, 2475]. Exposure to trinitrotoluene in ordnance plants has caused acute hepatic injury and postnecrotic cirrhosis [2519]. Atabrine has produced fatal chronic hepatic injury [677]. This list can be prolonged ad infinitum to include such substances as dinitrophenol, dinitrobenzene, and synthalin [2503]. Polyvinylpyrrolidone administration as a plasma expander leads to deposition of basophilic material in the liver, the functional significance of which is unknown [1119]. The effects of alcohol and tropical "toxic jaundice" are discussed in the chapter on nutritional liver damage, since they are largely the result of nutritional deficiency.

Idiosyncrasy. Sometimes hepatic injury follows drug administration without a clear-cut relation to the dose. Relatively small amounts are often responsible for these exceptional injuries. They are typical examples of iatrogenic diseases frequently catastrophic in nature and are often the bane of new types of therapy, since the unfavorable reactions are reported after considerable favorable experimental and clinical experience with a drug has been accumulated.

SULFONAMIDES. Sulfonamide administration leads to jaundice from liver damage in rare instances. This must be differentiated from hemolytic jaundice, also produced by sulfonamides, either directly or after sensitization. The effect of the underlying disease for which therapy is instituted is occasionally difficult to separate from the effect of the drug. Sulfanilamide is the most toxic; more recent sulfonamides, such as sulfasoxazole, are hardly ever toxic. Glucuronic acid conjugation is depressed in liver damage in favor of acetylation. The acetyl compounds of sulfadiazine or sulfasoxazole are more soluble than those of sulfanilamide or sulfapyridine, which makes the use of the newer drugs preferable in patients with liver disease [2977].

Sulfonamides in Liver Disease. In severe cirrhosis, side effects other than hepatic ones are common [2977], and renal injury is more frequent [1889]. In contrast, in hepatobiliary diseases in which a bacterial infection indicates the use of sulfonamides, hepatic function usually improves after therapy [1608, 2575]. Preexisting hepatic injury is therefore not necessarily a contraindication for sulfonamide therapy.

Sulfonamide Liver Damage without Liver Disease. Moderate damage by sulfonamides to a previously normal liver without clinical manifestations has been repeatedly suggested, but alterations in the results of hepatic tests have not been convincing [66, 3516]. The same is true of the demonstration of morphologic changes in the liver [2269, 3075], chiefly focal necrosis occasionally infiltrated with plasma cells [2786]. The appearance of jaundice from hepatic-cell damage, i.e., hepatitis, is the best proof of sulfonamide hepatotoxicity. This phenomenon is rare; 0.6 per cent of patients developed jaundice with earlier preparations [2056]. It is therefore an idiosyncrasy, unpredictable and not dependent upon the dose. Preceding liver damage is not a factor [227], nor are the duration of therapy and the interval between cessation of therapy and the appearance of jaundice. Nonfatal [1133] and fatal [227, 1467, 2342, 3703] reactions have been reported with almost all sulfonamides. The histologic appearance of the liver is one of fulminant massive necrosis [2342]. In some instances this resembles toxic hepatitis [2632]; in others, viral hepatitis [227, 1467]. In animal experiments hepatic injury has been produced [741], although the specificity of the lesion is questionable [2124], since a protective effect of sulfonamides against hepatotoxic injury has also been claimed [1046].

"Allergic Granulomas" from Sulfonamides. Sulfonamide therapy occasionally leads to allergic tissue changes with formation of hepatic granulomas [1093, 2342]. These changes often result from the underlying disease, especially in leukemia, in which the incidence of severe allergic reactions to sulfonamides is high [2042].

ANTIBIOTICS. The considerations concerning sulfonamides also apply to the antibiotics, except that fatal hepatic necrosis as a result of idiosyncrasy is extremely rare. Granulomas have been found in the liver following penicillin treatment [3519], but no change was seen in liver function except for reduction of stool and urine urobilinogen [1122]. Orally administered antibiotics in animals

afford protection against some hepatotoxic injuries [1327] and are possibly beneficial in human hepatic coma. Nevertheless, administration of larger doses produces liver damage in animals [1955]. Large doses of chlortetracycline intravenously produce jaundice [177, 2479], partly because of competition between excretion of the antibiotic and bilirubin [177] and partly because of hepatocellular damage [1954]. The drug inhibits several enzyme systems in the liver, particularly those concerned with oxidation [3719]. Furthermore, fatty metamorphosis frequently follows prolonged antibiotic therapy [2900], possibly as a result of a protein catabolic or antianabolic effect [974].

ANTITUBERCULOUS DRUGS. Isoniazid has been reported to produce toxic hepatitis with jaundice [2705], and Tibione to produce fatty liver [971].

ANTICONSULSANTS. Idiosyncrasy leading to hepatic necrosis is seen following treatment with phthienylate sodium [447, 2673], although in some instances viral hepatitis can not be excluded. Phenurone, which is apparently destroyed by the liver [3523], has produced fatal toxic hepatitis [1980]. Similarly, Dilantin [526] and Tridione [1920], as well as their combined use [851], have resulted in toxic hepatitis associated with damage to other organs.

Hepatic Side Reactions. In many drug reactions in which organs other than the liver are chiefly involved, toxic hepatic damage is encountered. Agranulocytosis produced by thiouracil [1132, 1537], propylthiouracil [632, 2035], or methimazole [2812] and exfoliative dermatitis following phenobarbital administration [3557] are often associated with toxic hepatitis. The hepatic damage following emetine therapy [1787] and the lesions associated with intoxications due to mercury, benzene, or various nephrotoxins are also side reactions.

Viral Hepatitis Mistaken for Toxic Reactions. Several conditions originally considered to be the results of drugs are now suspected to be viral hepatitis. Transmission via syringes probably accounts for the majority of instances of hepatitis which occur during antisyphilitic treatment with arsenicals or bismuth (see Syringe or Instrument-transmitted Hepatitis, under Epidemiology, Chap. 42). Jaundice which occurred during acriflavine therapy for gonorrhea belongs to the same group. Many instances of jaundice have been reported following cinchophen administration [333]. The erratic appearance of jaundice suggested that hypersensitivity is responsible, but many investi-

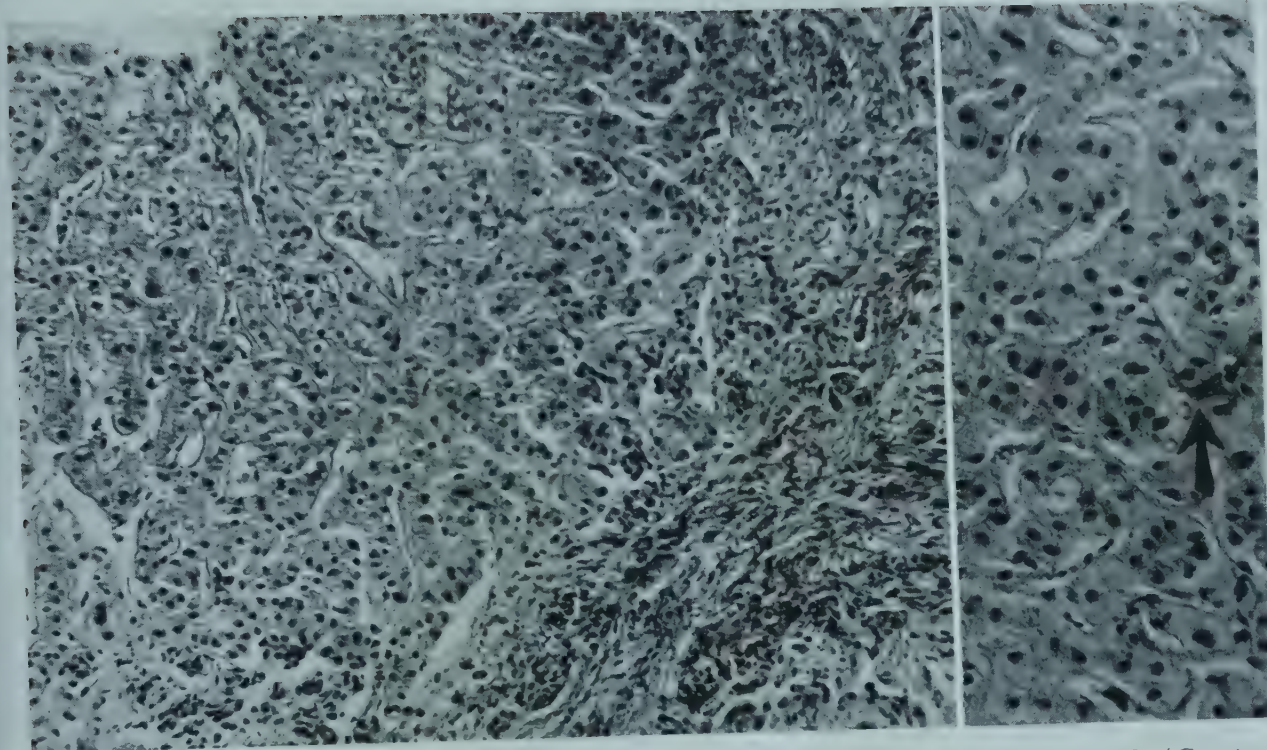


FIG. 133 Biopsy specimens in subacute cholangiolitis following arsenical treatment. H&E. (Courtesy of Dr. M. Stauffer.) *Left.* Normal arrangement of hepatic-cell plates and proliferation of peribiliary ductules surrounded by cellular exudate ($\times 100$). *Right.* Nearly normal appearance of hepatic cells. Bile canaliculi dilated and partly filled with bile plugs (arrow) ($\times 180$).

gators consider intercurrent viral hepatitis as the main cause.

ALLERGIC CHOLANGIOLITIS

Treatment with various drugs produces intrahepatic cholestasis with jaundice in a small number of persons. Total and prompt-reacting serum-bilirubin levels are elevated, and bilirubinuria is present, with increased serum-alkaline phosphatase activity and total serum-cholesterol levels. The results of the flocculation tests and other tests for hepatocellular degeneration are usually normal. In some instances skin lesions, positive results of patch tests, and blood and liver eosinophilia are found and are compatible with an allergic etiology. Histologically, mainly centrilobular cholestasis is seen, with bile pigmentation in the hepatic cells and Kupffer cells and with bile plugs in the bile canaliculi. This is usually associated with portal and periportal and often periductular infiltration of exudate containing some eosinophils. Therefore these lesions are designated as allergic cholangiolitis, using the term "allergic" in a broad sense. In some instances readministration of the offending agent after subsidence of jaundice does not result in recurrence of jaundice, while in other cases it does.

Arsenical Cholangiolitis

Intrahepatic cholestasis occurs as an unusual but well-defined complication of arsenotherapy [26, 1092, 1377, 2046, 3236]. In early stages eosinophilia is found and arsenical patch test results are usually positive. Serum-alkaline phosphatase activity and cholesterol levels are increased, and occasionally severe hypercholesterolemia and hyperlipemia result [3236]. Itching is prominent, but systemic manifestations are otherwise not severe. Histologically, periductular exudate is noted in addition to bile stasis (Fig. 133, left and right). Except for some feathery degeneration, the hepatic cells are normal. In protracted cases, portal fibrosis develops. Repeated injections of arsenicals sometimes produce gastrointestinal and other constitutional symptoms, followed in a few days by persistent jaundice associated with severe pruritus [1377].

Methyltestosterone Jaundice

Jaundice occasionally develops after administration of relatively large oral doses of methyltestosterone, but not after testosterone therapy [49, 397, 1767, 3559, 3654]. The jaundice is intense and usually lasts for many weeks without chills or fever. The serum-bilirubin level is elevated, and

bilirubinuria is common. Serum-alkaline phosphatase activity is occasionally increased, while the serum cholesterol is usually normal and the cholesterol ester ratio is occasionally reduced, the only laboratory evidence of hepatic-cell degeneration. In principle, the lesion is similar to other types of allergic cholangiolitis except that the portal and periportal inflammatory reaction is usually much less severe and bile stasis is in the foreground of the histologic picture. The lesion disappears eventually on discontinuation of the therapy and does not reappear if the drug is given again [3559]. If methyltestosterone is given to a jaundiced patient to relieve pruritus, the serum-bilirubin level may rise while the total serum cholesterol usually decreases [27, 2038].

Chlorpromazine Jaundice

Jaundice occasionally develops following the administration of chlorpromazine (Thorazine) [2048]; it apparently appears more frequently after oral therapy than after parenteral administration and is more frequent after prolonged use of the drug. Since jaundice may occur because of other reasons in patients receiving chlorpromazine, such as syringe-transmitted or serum hepatitis, the etiology is difficult to establish in individual cases. After exclusion of instances of viral hepatitis and extrahepatic biliary obstruction, a group of cases remains in which allergic cholangiolitis develops following chlorpromazine treatment. The jaundice occurs with a lack of malaise. Eosinophils are sometimes increased in the circulating blood. Serum-alkaline phosphatase activity is usually increased, and the serum-protein reactions are normal [1721]. Histologically, central bile stasis and pericholangiolitic exudate, frequently containing eosinophils, is noted. Hepatic-cell damage is minimal. Uneventful recovery seems to be the rule, although rare fatalities have been recorded. The major importance of the lesion lies in its resemblance clinically and in the laboratory to extrahepatic biliary obstruction [1721]. Therefore the possibility of allergic cholangiolitis must be considered in any patient following chlorpromazine treatment in whom surgical intervention is contemplated because of possible extrahepatic biliary obstruction. Liver biopsy sometimes permits a differentiation. Even if the differentiation is impossible and surgical exploration is required, additional procedures should be avoided if narrow extrahepatic bile ducts suggest intrahepatic cholestasis. Superfluous surgi-

cal procedures in such jaundiced patients may result in parenchymal injury and even death.

Jaundice Following Para-aminosalicylic Acid Treatment

Following prolonged administration of para-aminosalicylic acid (PAS), especially in tuberculosis, a hypersensitivity reaction may develop [478, 1995]. Patients with such a condition show increased serum-alkaline phosphatase activity, and on liver biopsy the picture of allergic cholangiolitis is found. In some instances jaundice develops, raising the differential diagnosis of infectious hepatitis or infectious mononucleosis. The picture of giant-cell hepatitis [1991] has also been reported after prolonged PAS administration, but whether the drug actually causes the lesion in such instances is questionable. Hypersensitivity reaction with jaundice has also been reported after administration of para-aminobenzoic acid [694].

NONSPECIFIC REACTIVE HEPATITIS

One of the most common morphologic alterations seen in liver biopsy specimens and found in many types of diseases is the combination of usually small areas of focal necrosis irregularly scattered throughout the lobular parenchyma with a mesenchymal inflammatory reaction in the lobule as well as in the portal tract. The lesion is produced by various toxins, including some poisons. It is also associated with many different infections, granulomas, tumors, and gastrointestinal diseases. It is found in surgical biopsy specimens as a result of the surgical procedure itself [1715]. In autopsy specimens, focal necrosis in the lobular parenchyma is less frequent, possibly because of suppression of the inflammatory reaction in the agonal period. The term "infiltrative hepatitis" has been recommended [2244], but in view of the non-specific nature of the lesion a more noncommittal term, "nonspecific reactive hepatitis," is proposed.

Clinical and Laboratory Findings. A definite clinical picture can not be associated with nonspecific reactive hepatitis, although in some instances it is the only anatomic finding that can be correlated with clinical manifestations referable to the liver. The liver is often enlarged, either by concomitant fatty infiltration, as in ulcerative colitis [1798] or occasionally in tuberculosis [2992], or by edema, particularly in acute infections [945, 1732, 2797]. Tenderness in the gall-

bladder region is a reflection of the associated edema in the gallbladder bed.

The correlation with laboratory findings is generally poor [1074, 2244], because the mild hepatic-cell damage with small areas of necrosis is associated with regeneration. The evaluation of the loss of function is therefore difficult. Nonspecific reactive hepatitis may be the cause of some of the abnormal results of the hepatic tests encountered in nonhepatic diseases. Serum-gamma globulin elevation is frequently associated with it, and in pulmonary tuberculosis, at least, the histologic alterations are correlated with an elevation of the serum gamma globulin [2907].

Structural Changes. The changes vary greatly in different livers and represent many of the abnormal reactions in the liver in a nonspecific fashion. In autopsy specimens the premortal and postmortal alterations, including central necrosis and edema, overshadow focal necrosis and portal inflammation. In biopsy specimens, diffuse mild hepatic-cell damage is indicated by variability in the size and appearance of neighboring cells and moderate degrees of regeneration, as well as by some irregularity in the arrangement of the hepatic-cell plates (Fig. 134E). Areas of focal necrosis are more dramatic in their appearance, although they only occasionally reach the size of half a high-power field and usually involve only a few cells with replacement of them by segmented leukocytes, monocytic cells, and other scavenger cells (Fig. 134A, B). Slight or moderate fatty metamorphosis is frequently seen. The Kupffer cells are proliferated and enlarged (Fig. 134C). They contain material engulfed by phagocytes, and their cytoplasm is often basophilic. Edema is rarely seen. The portal tracts are infiltrated with segmented leukocytes, lymphocytes, and histiocytes, which often contain pigment (Fig. 134D). Some tracts become enlarged, and some even assume the characteristics of a lymph follicle, but such changes are not uniform throughout the liver. Periportal and intralobular ductules are frequently proliferated and surrounded by some inflammatory exudate (Fig. 134F). The limiting membrane is usually intact.

If portal infiltration is in the foreground, the term "nonspecific reactive hepatitis" should be qualified by including portal inflammation in parentheses, whereas a predominance of intralobular changes should be indicated with the addition of intralobular inflammation. These differences are

not reflected in clinical or laboratory findings or in etiology.

Etiologic Factors. Nonspecific reactive hepatitis is a background for many other hepatic lesions discussed elsewhere. In biopsy specimens when other lesions, especially granulomas, are missed by the needle, such a nonspecific reactive hepatitis may be the only change found and may thus be misleading. Diseases in which focal involvement of the liver is in the foreground even in the presence of diffuse nonspecific changes are discussed under focal diseases, e.g., tuberculosis or sarcoidosis. At this point, the diseases in which only nonspecific changes are found are enumerated mostly on the basis of biopsy findings and sometimes only as a consequence of frequently abnormal results of hepatic tests.

GASTROINTESTINAL DISEASES. Nonspecific reactive hepatitis has been found in peptic ulcer [2279], gallbladder disease [179, 2244], ulcerative colitis [1798], and gastrointestinal carcinoma.

DIFFUSE SYSTEMIC DISEASES. The lesion has also been found in rheumatoid arthritis [2366], lupus erythematosus [1830], systemic nodular panniculitis [3191], and even in nonspecific febrile reactions [1232]. Serum-protein changes [1600] suggest impairment of liver function in rheumatic fever [1782] and rheumatoid arthritis [2414], although these changes do not necessarily result from hepatic dysfunction.

PNEUMONIA. Abnormalities of serum-protein reactions [3450, 3723] and carbohydrate metabolism have been reported in pneumonia. The serum-bilirubin level is often elevated, with an increase in the prompt-reacting fraction and with bilirubinuria and urobilinogenuria [926, 3723]. In lobar pneumonia involving the right lower lobe, jaundice was seen in the era before antimicrobial therapy; it is now rare [2920]. Jaundice previously implied a bad prognosis [2920, 3723], but little relation is now found between the severity of pneumonia and the degree of jaundice [1759]. Malnutrition increases the frequency and intensity of jaundice in pneumonia. Increased hemolysis from local blood destruction has been considered a cause of the jaundice [943], but the results of hepatic tests implicate hepatocellular impairment as the main factor [3723]. This impairment has been related to anoxia [2748], although this is unconfirmed by clinical observations [1759], to a nonspecific alarm reaction [3723], and to toxemia. Morphologically focal necrosis and portal inflammatory changes are

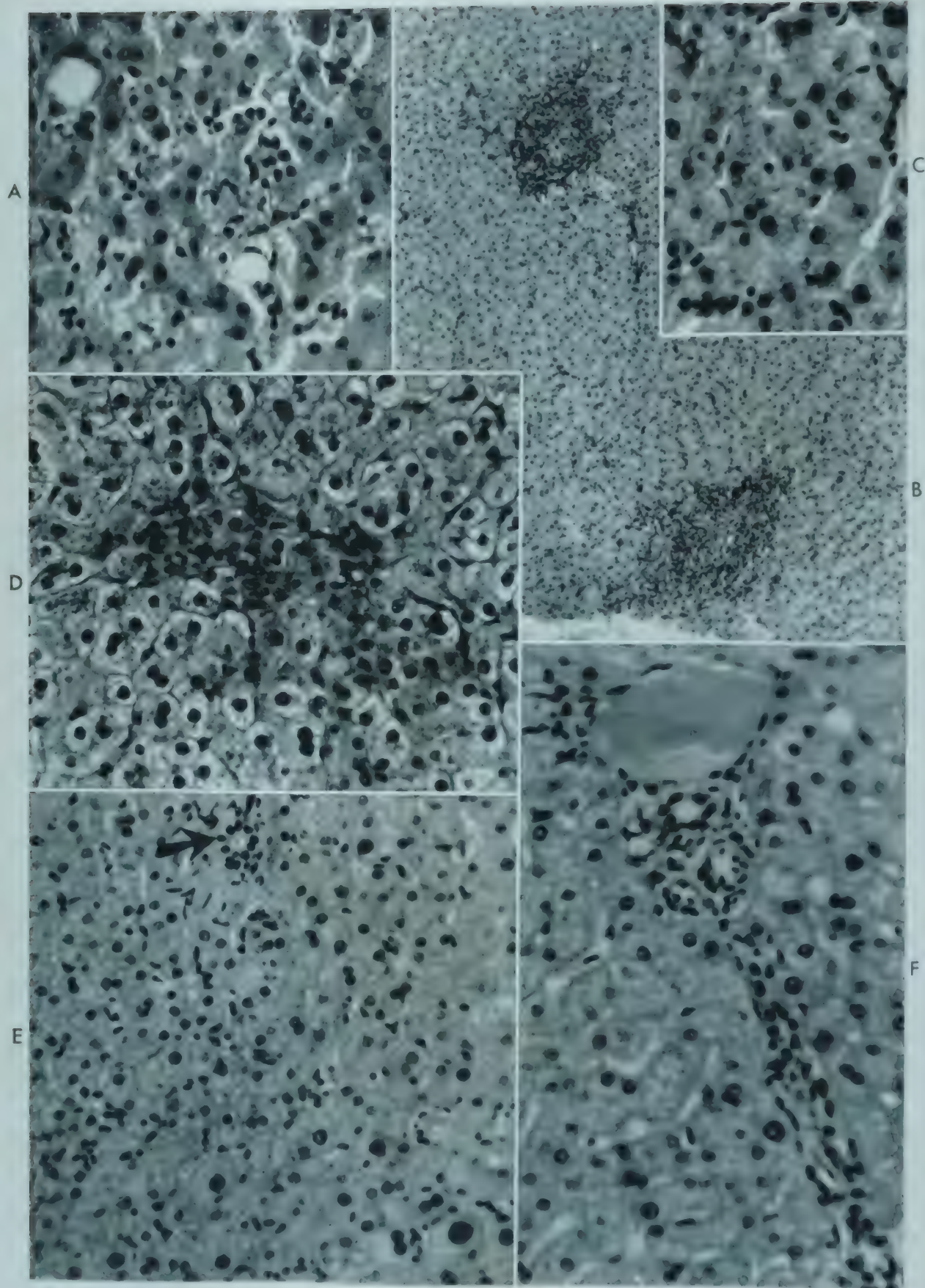


FIG. 134 Biopsy specimens showing various features of nonspecific reactive hepatitis. H&E. *A*: Focal necrosis with replacement of hepatic cells by round cells and segmented leukocytes ($\times 220$). *B*: Extensive portal infiltration, mainly by mononuclear cells. Multiple small areas of focal necrosis and proliferation of Kupffer cells are also seen ($\times 70$). *C*: Proliferation of Kupffer cells ($\times 270$). *D*: Accumulation of mononuclear cells and segmented leukocytes in small portal tract ($\times 220$). *E*: Irregular arrangement of hepatic cell plates with circumscribed regeneration, including multinucleated cells, areas of focal necrosis, and accumulation of exudate cells around intrahepatic ductule (arrow) ($\times 125$). *F*: Increase in peribiliary ductules surrounded by exudate cells ($\times 240$).

common. In autopsy specimens centrolobular necrosis is seen in addition [943, 1801]. Sometimes the necropsy findings are surprisingly meager in fatal pneumonia with jaundice.

"BACTERIAL HEPATITIS." In coccal infections other than pneumonia, hepatic involvement is less frequent. The term "streptococcus hepatitis" [1847, 2154], previously used, is now seldom applied, since many instances were probably viral hepatitis. Jaundice in septicemia seems to be hemolytic rather than hepatocellular [2503]. Bacterial hepatitis is seen in extrahepatic biliary obstruction which has become infected via ascending, hematogenous or lymphogenous routes (see Infected Biliary Hepatitis, Chap. 47) and in suppurative pyelophlebitis (see Suppurative Pylephlebitis, under Diseases of the Portal Vein, Chap. 56). Gas bacillus infections of the liver produce jaundice but no well-defined hepatic lesion [2503].

Umbilical Sepsis in the Neonatal Period. This is a form of bacterial hepatitis. Symptoms usually start in the first week of life, but jaundice becomes noticeable only shortly before death. The umbilical wound is frequently not conspicuous. In the liver portal and periportal inflammation is noted, with many segmented leukocytes, occasionally progressing to abscess formation. Large focal or zonal areas of necrosis may also be found, probably as a result of vascular obstruction [2347, 2650].

Hepatic Changes in Subacute Bacterial Endocarditis. In subacute bacterial endocarditis, proliferation of Kupffer cells is the most prominent histologic change in the liver (Fig. 34, upper left). The degree of congestion depends on the severity of heart failure. Portal inflammation, hepatic-cell degeneration, and focal necrosis are similar to such conditions in the other diseases enumerated.

"*Gonococcal Hepatitis.*" Instances of jaundice during gonococcal infections appear to be serum hepatitis transmitted during treatment, rather than the result of a specific effect of the bacteria. Low-grade diffuse peritonitis spreading from gonorrheal salpingitis is sometimes localized around the gallbladder. It heals with adhesions, which glue the organs to the colon or other surrounding viscera. The resulting fibrotic pericholecystitis causes pain in the gallbladder region, especially during contraction of the organ. On x-ray examination contraction of the gallbladder after a fat meal produces a characteristic tentlike picture.

"*Salmonella Hepatitis.*" The hepatic lesion produced in typhoid fever is the best studied among the *Salmonella* group. Jaundice sometimes occurs.

Aside from nonspecific reactive hepatitis, three abnormalities are commonly found in autopsy specimens: (1) small areas of focal necrosis; (2) portal infiltration by lymphocytes, plasma cells, mononuclear cells, and histiocytes, some of which contain engulfed material; (3) the typhoid granuloma, which develops by proliferation of Kupffer cells and accumulation of large mononuclear cells in the sinusoids. After approximately 2 weeks, the obstruction of the sinusoids results in central necrosis. In the third week [2187] typhoid bacilli are found in the bile. Cholangitis occurs exceptionally. Cholecystitis during the recovery period and even years later, with typhoid ulcers in the gallbladder wall, is not rare. Similar hepatic lesions are seen in the various other salmonella infections. As a rule, the intralobular granuloma is less common than focal necrosis and portal inflammation, except in *Salmonella typhimurium* infections, in which this lesion is outspoken. Jaundice is more common than in typhoid fever, as a result of diffuse parenchymal changes rather than focal alterations [1239]. Cholangitis may develop.

GAS BACILLUS INFECTIONS. Gas bacillus infections of the liver produce jaundice but no circumscribed lesion [2503].

RICKETTSIAL DISEASES. In epidemic and scrub typhus and in Rocky Mountain spotted fever, nonspecific hepatitis is found [41]. This includes foci of necrosis of varying sizes, containing segmented leukocytes and other mesenchymal cells, especially in epidemic typhus and Rocky Mountain spotted fever. Proliferation of the Kupffer cells is noted, with erythrophagocytosis and cytophagocytosis. Mononuclear elements are increased in the sinusoids as well as in the portal tracts. Furthermore, phlebitis and arteritis may develop as part of the hyperergic reaction characteristic of this disease. Finally, central necrosis, apparently related to shock, appears. Various functional changes have been reported [3645].

VIRAL DISEASES. In poliomyelitis, focal necrosis occurs in the liver. In smallpox, areas of focal necrosis and severe Kupffer cell reaction have been observed [380]. Similar findings have been reported in generalized vaccinia [1352].

TOXIC HEPATIC NECROSIS

Toxic hepatic necrosis is a hepatic disease characterized mainly by zonal necrosis (usually central) which often becomes massive or submassive. The etiologic factors are well-defined poisons in

only a few instances—about 30 per cent in our own material [2632]. In most instances, the responsible noxious substances can not be elicited, and sometimes endogenous toxins are suspected.

Clinical Course. The differentiation of toxic hepatic necrosis from viral hepatitis is clinically, functionally, and even anatomically difficult if the history fails to give a clue. The prodromal symptoms are usually short and without the features of gastrointestinal or upper respiratory disease seen in viral hepatitis. In some instances vague symptoms such as malaise, bloating, and tenderness of the liver precede the development of jaundice by weeks. After jaundice has developed, the patients appear very sick, often more sick than in viral hepatitis. Vomiting and severe lassitude are common. Jaundice is deep, and a hemorrhagic diathesis is found in severe cases. The liver is usually enlarged, and shrinkage of the liver is unusual even in fatal cases. The spleen is not palpable in the acute stage. Because of the toxic etiology of the disease, changes are found in other organs. Furthermore, when the necrosis is associated with fatty metamorphosis, increased fat deposition is found elsewhere, such as in the heart, kidneys, and pancreas. Oliguria, uremia with frost, and signs of acute toxic nephrosis occur, as do bradycardia, prolongation of the QRS complex, and dependent edema because of cardiac involvement.

The mortality rate is relatively high. In exceptional instances protracted hepatic failure with or without jaundice follows an episode of acute toxic hepatitis. After a year or less, cirrhosis develops. The laboratory findings are those of severe hepatic-cell degeneration, with the results of the flocculation tests normal in 30 per cent of the cases [2632]. In acute chemical hepatitis they are frequently normal. The cholesterol ester ratio in serum is depressed, and cholinesterase activity drops precipitously. Cholestasis with increase of alkaline phosphatase activity is common, as is azotemia.

Structural Changes in Biopsy Specimens. In liver biopsy specimens, central necrosis and disappearance of hepatic cells are found [2632] (Fig. 136A). In milder forms, the hepatic cells in the central portion are distinctly acidophilic, and only isolated cells have disappeared. In more severe instances anuclear cell fragments are seen. The central zone is frequently infiltrated by segmented leukocytes, whereas the portal tracts appear normal or contain inflammatory cells, which are also found around ductules (Fig. 136B). Sometimes diffuse fatty metamorphosis is noted.

Macroscopic Changes. In fatal toxic hepatic necrosis, the liver is larger than normal, especially when fat is abundant [1779], averaging 1,800 gm [2632]. The anterior edge is blunted, and on the cut surface the lobular architecture is exaggerated because the necrotic central zones are red and depressed (Fig. 135A). In places, red bridges connect adjoining central fields and thus surround the remnants of the parenchyma on the periphery of the lobule. The appearance simulates subacute passive congestion, from which it can be differentiated by the decreased consistency of the liver. If the fat content is increased, the lobular pattern is obscured, but the consistency of the liver is still diminished, despite its doughy character. The disease process is uniform throughout the liver on the cut surfaces, and no differences are noted between the lobes. The gallbladder is dilated, and the gallbladder bed is often edematous as an expression of a generalized hepatic edema (Fig. 135C). The lymph nodes at the porta hepatis are seldom enlarged.

Histologic Alterations in Autopsy Specimens.
CENTRAL NECROSIS. The most common histologic change is necrosis with or without disappearance of the hepatic cells in the central and intermediate zones (Fig. 135B). Occasionally necrosis extends to the portal tract (submassive necrosis), or it involves the entire lobule (massive necrosis). When massive or submassive necrosis is widespread, as in sulfonamide idiosyncrasy, an "acute atrophy" results which is indistinguishable from the same lesion in viral hepatitis. The extent of the necrosis seen in the autopsy specimen does not mirror the clinical or functional findings [1996]. Sometimes, only a peripheral rim is intact where the hepatic cells appear normal, contain glycogen, and are regenerating. Probably the necrosis is significantly enlarged in the agonal period. Fatty metamorphosis in the central zone is fairly common (Fig. 135D), and in some instances it is diffuse and even precedes the development of necrosis.

In the process of necrosis, relatively large anuclear hepatic-cell fragments or remnants form (Fig. 136E). If they persist, the intermediate zone is relatively wide. Segmented leukocytes accumulate around them. If the fragments have disappeared, protein-rich exudate remains in the denuded central and intermediate zones, with a few inflammatory cells, mainly histiocytes, engaged in phagocytosis (Fig. 136C). In addition sinusoids and tissue spaces are packed with erythrocytes.

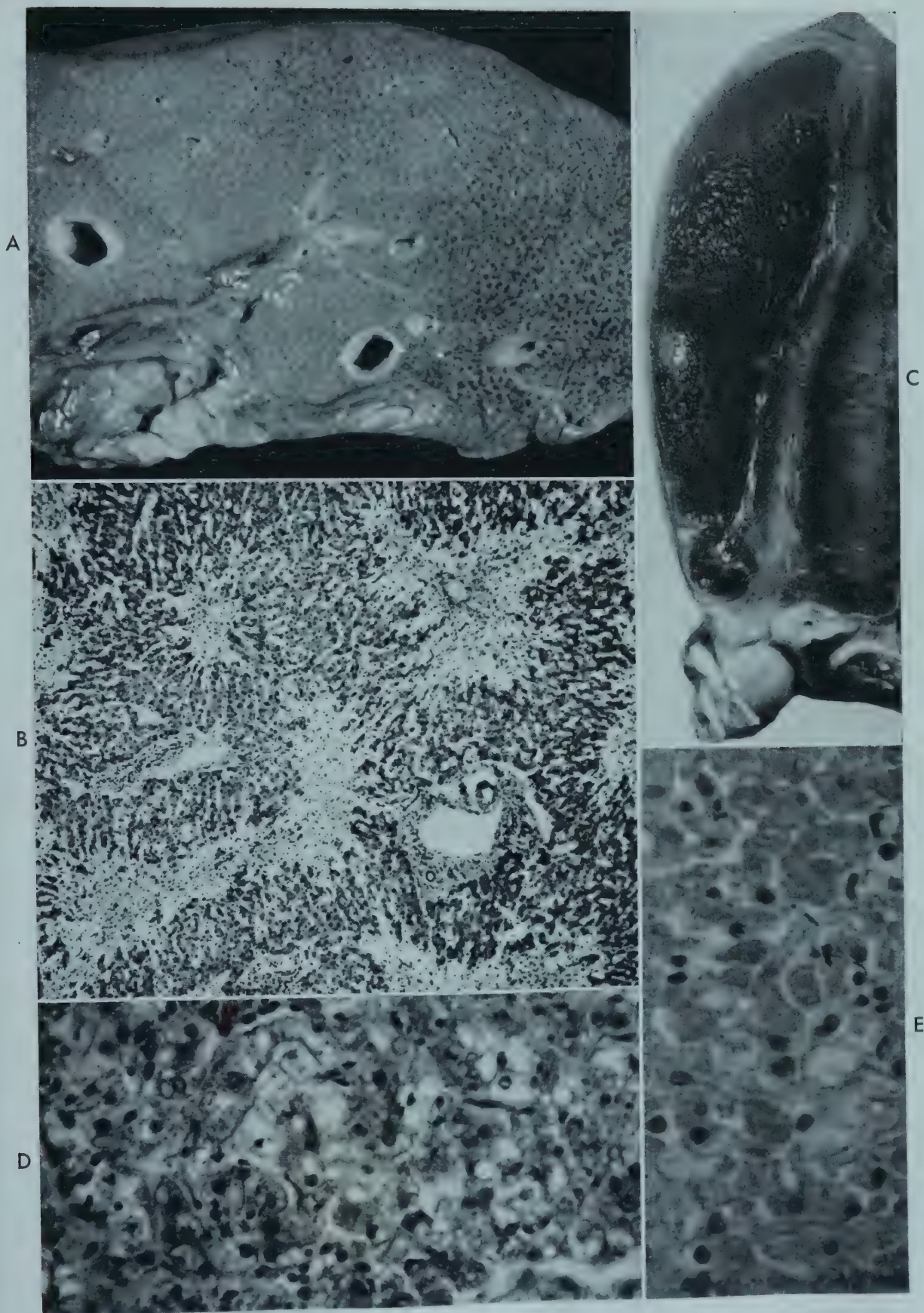


FIG. 135 A. Acute toxic necrosis from carbon tetrachloride ingestion. Note exaggerated lobular architecture. B. Disappearance of hepatic cells in center of lobule, with collapse of framework after acute carbon tetrachloride intoxication. H&E ($\times 60$). (Popper, H., Steigmann, F., Meyer, K. A., Kozoll, D. D., and Franklin, M.: *Am.J.Med.* 6:278, 1949.) C. Gallbladder bed edema following severe burn. (Eppinger, H., Kaunitz, H., and Popper, H.: *Die seröse Entzündung*, Berlin, Springer, 1935.) D. Degeneration of fatty hepatic cells in carbon tetrachloride poisoning. H&E ($\times 220$). E. Coagulation necrosis of hepatic cells in the center of lobule in creosote intoxication. H&E ($\times 220$).

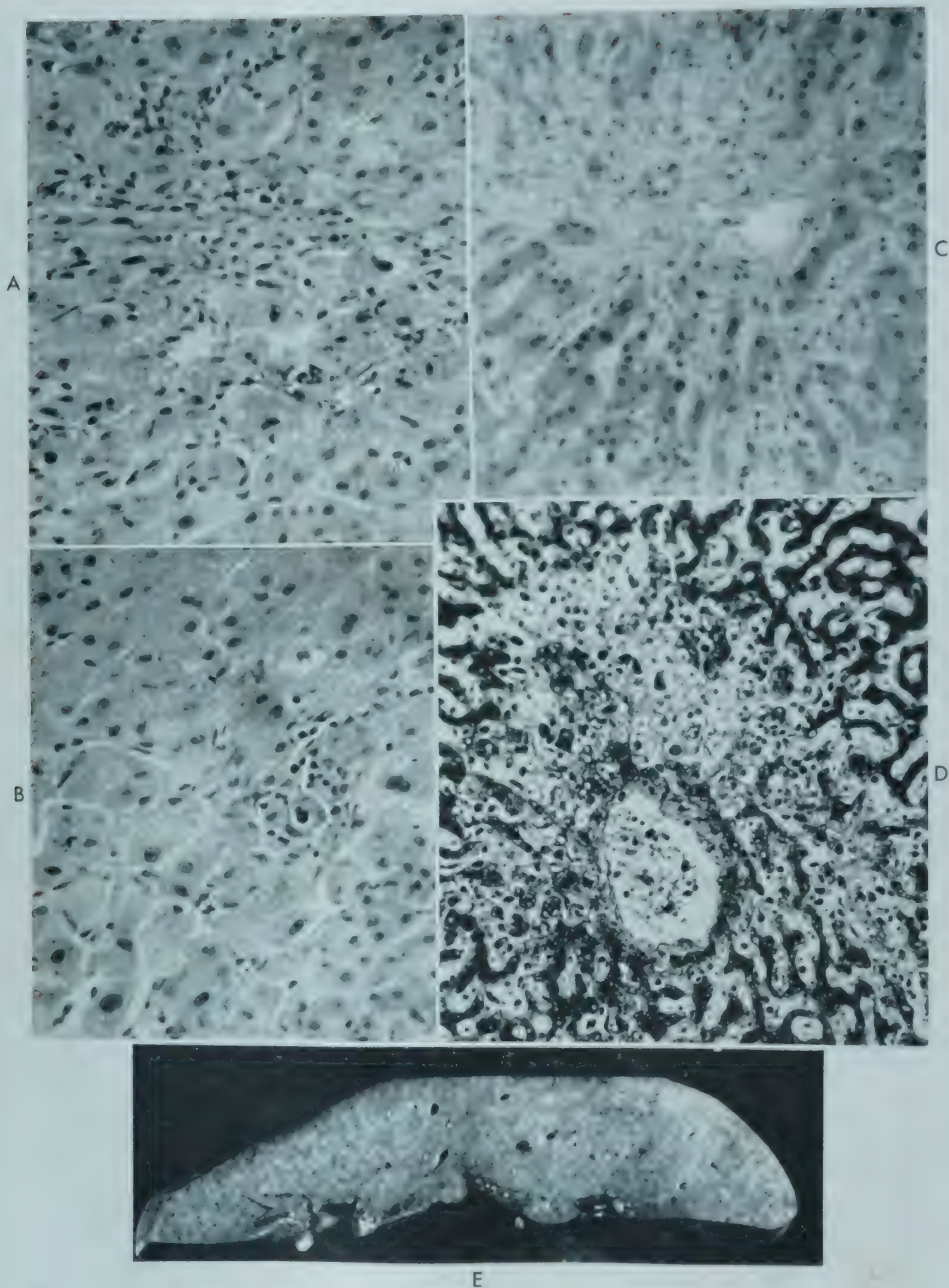


FIG. 136. A. Liver biopsy specimen in carbon tetrachloride intoxication. Central necrosis with accumulation of a few exudate cells. H&E ($\times 220$). B. Same as A. Focal necrosis and accumulation of exudate cells around intralobular ductule. H&E ($\times 220$). C. Disappearance of necrotic hepatic cells in the central zone and minimal inflammatory reaction. H&E ($\times 220$). D. Centrilobular necrosis around contracted central vein in premalignancy. H&E ($\times 125$). E. Cirrhosis following chronic trinitrotoluene (TNT) intoxication.

The central and sublobular veins appear contracted, and the piercing veins are very narrow (Fig. 136D). Rupture of the reticulum fibers, which occurs infrequently, is recognized by extensive blood pools. The hepatic cells bordering on the necrotic area show ramified clumping of the cytoplasm, which fills only part of the cell, similar to the hyaline bodies of Mallory found in alcoholic cirrhosis [2187, 2337, 2807]. In the intact zone of the lobule, bile stasis is noted and edema is severe. Bile duct and ductular proliferation is occasionally prominent. Intralobular ductules containing bile casts are rare. The portal tracts are not infiltrated in earlier stages. Later, histiocytic cells accumulate, and some of them contain pigment.

In later stages, the framework collapses, and the original central and intermediary zones shrink. The portal tracts are enlarged and stellate-shaped. They are infiltrated with mononuclear cells and contain pigment. Proliferation of bile ducts and ductules is prominent. Eventually central collapse leads to replacement fibrosis, and connective tissue membranes radiate into the intact parenchyma. Some also radiate from the portal tract. Eventually the lobular architecture is obscured but not destroyed.

PERIPHERAL NECROSIS. Extensive necrosis of the hepatic cells on the periphery of the lobule is relatively rare, having been found only once in a series of 69 cases [2632]. Denuded peripheral connective tissue makes the portal tracts appear much larger. Leukocytic infiltration is present, and bile duct proliferation is common. In the few cases studied, no etiologic factor was demonstrated. The literature records peripheral necrosis in phosphorus and mushroom poisonings. In phosphorus poisoning, usually with a short survival time, fatty infiltration begins at the periphery and proceeds until the entire liver becomes involved, and cell necrosis is also usually peripheral, in contrast to what occurs in most other poisonings [1470]. Similar lesions supposedly result from other poisons [1470].

MID-ZONAL NECROSIS. Mid-zonal necrosis is rarely seen now on a toxic basis. The earlier literature records such findings in delayed chloroform poisoning, especially in obstetrical patients with prolonged labor [2817, 3169]. In animal experiments, it occurs in toxic injuries in an otherwise damaged liver [294]. It has also been found after trauma.

Cirrhotic Transformation. Chronic cirrhotic stages are rare, or at least etiologically not well

established, because the toxic insult must either persist or be repeated after short intervals before recovery to lead to cirrhosis. Cirrhosis has been seen after mushroom or trinitrotoluene intoxication (Fig. 136E).

The lobular architecture is obscured, with formation of nodules of different sizes, which produce a hobnail appearance on the surface of the liver. Some nodules are large and consist of several lobules. Wide scars are found as remnants of massive necrosis. The wide scars composed of ghost lobules seem to differ from those found in viral hepatitis by the smaller numbers of pigmented large histiocytes and the less prominent ductular proliferation. The status of the hepatic cells in the preserved lobular parenchyma and in the nodules varies. In hepatic failure, severe damage is noted, characterized by partial coagulation necrosis centrally as well as peripherally, usually associated with severe cholestasis.

"Acute Yellow Atrophy." The term "acute yellow atrophy" was first applied by Rokitsansky to toxic hepatic necrosis in instances of shrunken, distinctly yellow livers as a result of phosphorus poisoning and therefore associated with fatty metamorphosis. This pathologic entity was better known in the past than it is now. Such livers represent instances of massive necrosis in the greater part of the liver, which today is mainly encountered in viral hepatitis, in which the color is less yellow and more red (see Massive Necrosis, under Structural Alterations, Chap. 43). Chloroform poisoning [126, 1470, 2554], still found in children on occasion, and mushroom intoxication [1470, 1801] also produce acute massive necrosis of the liver. The intoxication in these instances seems to be very rapid, but the clinical features are not different from those in other forms of toxic hepatic necrosis except for the clinically demonstrable shrinking of the liver.

Postoperative Toxic Hepatitis. **CLINICAL FEATURES.** A few days after surgery, especially laparotomy, a rapidly deepening jaundice develops occasionally in patients who did not necessarily exhibit functional evidence of hepatic damage before operation. The liver is enlarged and tender. Hepatic coma develops, and the patient dies within a few days following the onset of jaundice. The results of the flocculation tests remain normal, alkaline phosphatase activity and serum NPN are usually increased.

STRUCTURAL ALTERATIONS. At the autopsy, the large green liver shows exaggerated lobular archi-

texture and gallbladder bed edema. Histologically, edema, central necrosis, and dilatation of bile canaliculi and ductules filled by bile plugs are seen. Little inflammatory exudate is noted in the portal tracts.

PATHOGENESIS. The functional and structural findings suggest hepatocellular damage, intrahepatic cholestasis, or cholangiolitis, and hemolysis. The following factors apparently contribute to the

development of the lesion: (1) tissue breakdown, since the extent of the operation seems to be of importance; (2) anoxia, possibly as a result of anesthesia; (3) postoperative shock; (4) anemia owing to blood loss; (5) blood transfusions (see Posttransfusion Hepatitis, under Epidemiology, Chap. 42). The contribution of these different factors varies in individual cases, providing a combination of toxic, anoxic, and circulatory effects.

HEPATIC INJURY FROM INFECTIOUS AGENTS: CLASSIFICATION; ETIOLOGY AND EPIDEMIOLOGY OF VIRAL HEPATITIS

Many microorganisms damage the liver, but most of them produce only focal injuries. Those producing diffuse injury involve other organs simultaneously, and only a few are distinctly hepatotropic, causing a primary liver disease. Nevertheless, inclusion or exclusion of diseases in this chapter on diffuse hepatic injury from infectious agents is arbitrary. Many primarily focal diseases such as hepatic amebiasis, syphilis of the liver, and sarcoidosis have diffuse forms which are primary liver diseases. This group is not discussed in this chapter but is described under the granulomatous diseases and parasitic infestations because of differential diagnostic considerations. Other diseases from infectious agents in which the host reaction is in the foreground are discussed under nonspecific reactive hepatitis.

Classification of Hepatic Injury from Infectious Agents

Viruses

Viral hepatitis

Infectious mononucleosis (see Hepatitis Caused by Infectious Mononucleosis, Chap. 45)

Yellow fever (see Yellow Fever Hepatitis, Chap. 45)

Rift Valley fever (see Rift Valley Fever Hepatitis, Chap. 45)

Herpes simplex hepatitis (see this heading, Chap. 45)

Vaccinia (see Viral Diseases, under Nonspecific Reactive Hepatitis, Chap. 41)

Smallpox (see Viral Diseases, under Nonspecific Reactive Hepatitis, Chap. 41)

Poliomyelitis (see Viral Diseases, under Nonspecific Reactive Hepatitis, Chap. 41)

Viral hepatitis in animals (see this heading, Chap. 45)

Rickettsia (see Rickettsial Diseases, under Nonspecific Reactive Hepatitis, Chap. 41)

Bacteria

Streptococcal infections (see "Bacterial Hepatitis," under Nonspecific Reactive Hepatitis, Chap. 41)

Pneumococcal infections (see Pneumonia, under Nonspecific Reactive Hepatitis, Chap. 41)

Gonococcal infections (see "Gonococcal Hepatitis," under Nonspecific Reactive Hepatitis, Chap. 41)

Salmonella infections and typhoid fever (see "Salmonella Hepatitis," under Nonspecific Reactive Hepatitis, Chap. 41)

Gas bacillus infections (see Gas Bacillus Infections, under Nonspecific Reactive Hepatitis, Chap. 41)

Tuberculosis (see Hepatic Tuberculosis, Chap. 54).

Tularemia (see Tularemia, under Hepatic Granulomas in Various Diseases, Chap. 54)

Brucellosis (see Hepatic Brucellosis, Chap. 54)

Leprosy (see Leprosy, under Hepatic Granulomas in Various Diseases, Chap. 54)

Syphilis (see Hepatic Syphilis, Chap. 54)

Relapsing fever (see Relapsing Fever, Chap. 45)

Leptospirosis; Weil's disease, Canicola fever (see Leptospiral Hepatitis, Chap. 45)

Fungi

Histoplasmosis (see Histoplasmosis, Chap. 54)

Actinomycosis (see Actinomycosis, Chap. 54)

Blastomycosis (see Other Fungus Infections, Chap. 54)

Parasites

Amebiasis (see Hepatic Amebiasis, under Protozoan Infestations of the Liver, Chap. 55)

Malaria (see Malarial Hepatitis, Chap. 45)

Kala-azar

Trypanosomiasis (see Trypanosomiasis, Chap. 45)

Cestode infestation (see Segmented Flatworms—Cestodes, Chap. 55)

Nematode infestation (see Roundworms—Nematodes, Chap. 55)

Trematode infestation (see Nonsegmented Flatworms—Trematodes, Chap. 55)

Arthropod infestation (see Arthropods in the Liver, Chap. 55)

VIRAL HEPATITIS—ETIOLOGY AND EPIDEMIOLOGY

Two viruses are now recognized as being responsible for viral hepatitis [1420, 2409, 3230]. One (IH or A), which causes the infectious or epidemic form, is primarily water- or food-borne; the other (SH or B), which causes serum hepatitis, or homologous serum jaundice, is parenterally transmitted. Although differences between the viruses are fully established, differences in the clinical, laboratory, and pathologic findings have not been convincingly demonstrated, although some investigators have claimed that they can be demonstrated [2409].

Hepatitis of viral etiology is the most important primary hepatic disorder, at least in the United States. Although the infectious nature of this condition was known, having been demonstrated especially by epidemics during wars or outbreaks in institutions, it is only during and following World War II that the viral etiology has been confirmed, primarily on the basis of studies with human volunteers. The mechanism of action of the viral effect is not clear. The morphologic appearance suggests infection of scattered hepatic and ductular cells or even of all epithelial cells. The reaction of the mesenchyme is probably secondary to the epithelial invasion. Some of the changes, such as the hepatic eosinophilia, possibly represent an allergic reaction to viral products.

Variations of the basic lesion account for different forms, degrees, and stages. The most severe form was originally called "acute yellow atrophy," although the designation "yellow" is rarely appropriate, since the color of the liver is usually red or brownish-green (see Massive Necrosis, under Structural Alterations, Chap. 43). The term remained in general use until World War II, when it was replaced by "fatal or fulminant epidemic hepatitis" [2083, 2085]. Milder forms were orig-

inally called "catarrhal jaundice," in deference to the classic but incorrect theory of Virchow, according to which catarrhal inflammation of the duodenum encroached upon the common bile duct and caused its obstruction by a mucous plug. Necropsies performed in World War I on soldiers killed while sick with catarrhal jaundice finally confirmed the opinion that this disease is a primary hepatocellular disorder [943] and a milder form of "acute yellow atrophy."

Epidemiologic observations in infants and adults, as well as extensive screening studies first made during World War II, indicate that an anicteric form of viral hepatitis exists and that it seems to be widespread. Similarly chronic forms are not necessarily associated with jaundice.

Etiology

Properties of the Virus. **CULTURE.** After various unsuccessful or unconfirmed attempts abroad [213, 2558, 3065] and in this country [618], two strains of hepatitis present in serums or stool suspensions were propagated in minced chick embryo cultures or rabbit liver tissue cultures [1461]. From these cultures the disease was successfully transmitted to human volunteers, although they did not become jaundiced [829].

RESISTANCE. The virus is relatively heat-resistant, withstanding temperatures of 56°C for 30 minutes [1420]. Heat treatment at 60°C for 10 hours, as used in the production of human serum albumin, inactivates the virus [1145], although 4 hours of treatment at 60°C is insufficient [2388]. Cold ethanol fractionation used in the production of human immune globulin apparently also inactivates the virus [2390]. It survives for at least 1 year at 10 to 20°C [2409]. It withstands the usual disinfectants such as alcohol or mercurial antiseptics. Chlorination of water does not inactivate the virus [2418]. Only autoclaving and dry heat are reliable methods for sterilization. Ultraviolet irradiation has been suggested as an efficient method of eradicating the virus [300, 2478], but hepatitis has resulted from transfusion of irradiated plasma [1631, 2826, 2894]. Treatment with nitrogen mustard has also been ineffective [828]. Sulfur, alkyl oxides, and beta-propiolactone are now being studied as sterilizing agents. A combination of ultraviolet irradiation and treatment with beta-propiolactone appears most promising [1404]. Storage of plasma at room temperature attenuates the virus and decreases the incidence of serum hepatitis [44, 46, 2391].

SIZE. The virus is very small, 26 m μ or less, as judged from passage through filters of known pore size [2109]. Attempts to demonstrate the virus by morphologic methods have been unsuccessful [213]. Inclusion bodies in nuclei are seen [2441], but whether they are the virus is doubtful. The acidophilic degeneration of the cytoplasm resulting in "Councilman body" formation has been considered a specific reaction of the cell to the virus, in the sense of cytoplasmic inclusion bodies [213]. Such bodies are found in other hepatotropic virus diseases, such as yellow fever or canine hepatitis, but their specificity is still questionable.

Serologic Reactions and Skin Tests. Specific serum reactions based on precipitins have not been developed [213]. Antibodies have been demonstrated in viral hepatitis, but their specificity is questionable [874, 956, 1423]. Contrary to earlier reports, titers of cold or heterophil agglutinins or Kahn test titers do not vary significantly in hepatitis [1425].

A skin test for A virus prepared from amniotic fluid of inoculated embryonated hens' eggs produces positive reactions in persons with histories of spontaneous or induced A virus hepatitis, while in B virus hepatitis the incidence of positive tests is not higher than in random cases [1460]. The skin test has been useful in epidemics [218]. The possibility that living virus is present in the antigen is suggested by immunity imparted by skin testing [831]. The skin test material is still not standardized and is effective only in very high concentration [3230]. The results of skin tests suggest that A virus is one single antigenic entity, whereas B virus seems to be composed of several different strains with little cross immunity [3230].

Animal Transmission. Transmission of the virus to animals has not been possible, despite some reports to the contrary in foreign literature [498, 2103, 2558], none of which has been confirmed [618]. Also unconfirmed are attempts to transmit the disease to animals with altered resistance, such as rats on protein-deficient diets [2103], rats under intensive cortisone treatment [2258], or mice treated with urethane [1660]. In the latter two instances, an infectious agent that produced hepatic changes was transmitted by repeated passages, but whether this is the human hepatitis virus or another microorganism is unknown. The failure to transmit human hepatitis to any animal makes the animal kingdom an unlikely reservoir for human hepatitis, although various types of animal hepatitis are known.

Transmission to Human Volunteers. In view of the difficulties in identifying the virus by animal transmission, serologic reactions, or skin tests, the transmission to human volunteers remains the only method of demonstrating the virus and of differentiating A virus from B virus. This transmission has been repeatedly performed since 1942 [1012, 1420, 2101, 2409, 3426], and over 1,500 such instances are now recorded [3230].

Depending upon the source of the inoculum, a variable percentage of volunteers, seldom approaching 50 per cent, develops jaundice with clinical manifestations and laboratory findings of hepatitis [827, 833, 1420, 1431, 2420]. Other volunteers develop clinical and laboratory findings of hepatitis without jaundice. Serum and stools are infective for A virus hepatitis when administered either orally or parenterally. Volunteers infected parenterally with A virus excrete it in the feces [1420]. In contrast, B virus can not be transmitted via fecal extracts or by oral administration of serum [1420, 2100, 2477, 3230]. Nasal washings have not been found infective in either A or B virus hepatitis [2101, 2413], and similarly urine has not been convincingly proved to be infective [1012, 1420, 3230]. The duration of infectivity in serum or feces of the viruses is still the subject of argument. The serum contains A virus shortly before and for a short time after the appearance of jaundice [1062, 1420].

The Carrier State. Investigations on human volunteers established the carrier state for both A and B hepatitis virus. In the carrier state clinical symptoms and a history of jaundice are absent, although hepatic function and structure may be abnormal [2389, 2415, 3231]. The virus may be transmitted (1) by persons who never had the disease and whose serum harbors the virus; (2) by persons who have completely recovered from the disease and now carry the virus in their blood without any alteration of the liver; (3) by persons who have a persistent, mild, anicteric hepatitis with permanent or sporadic viremia. The last possibility suggests that donors should be screened, with laboratory tests used to reveal hepatic damage.

VIRUS A CARRIERS. Feces, serum, and liver biopsy specimens from human volunteers in whom infectious hepatitis was produced with A virus and in whom symptoms persisted for 9 to 12 months, produced vague clinical symptoms without jaundice and without significant laboratory findings when inoculated into other volunteers [2419]. The

A virus was found in the stools of two children with chronic hepatitis without jaundice for more than a year during an epidemic [218]. Therefore, fecal carriers probably exist before, during, and after infections.

VIRUS B CARRIERS. Parenteral inoculation of human volunteers with serum hepatitis from patients in various stages of the disease causes abnormal results of hepatic tests occasionally in the incubation period and in the acute preicteric phase but not during convalescence [1420, 2100, 2477]. In the extensive search for the healthy-appearing donors who transmit serum hepatitis, a series of persons has been found from whom the virus has been successfully transmitted to volunteers [2409, 3230]. In some of these carriers abnormal results of hepatic tests and abnormal biopsy findings were obtained [3231]. Abnormal results of one or more hepatic tests, especially the thymol turbidity test, were noted in the donors of blood the recipients of which developed hepatitis [1026], and non-specific histologic changes were present in biopsy specimens of such recipients [2415]. Moreover, when their blood was given to volunteers, viral hepatitis was produced [2389]. These studies are

evidence of an asymptomatic blood carrier state for B virus, possibly the result of failure of the host to attain immunity early in the infection [3231] or the result of transplacental transmission without structural and functional changes.

SCREENING FOR CARRIERS. Carriers without structural or functional alterations can not be detected by any screening methods. However, some carriers have abnormal hepatic function and structure because of viral hepatitis infection without an acute symptomatic stage. This infection may even progress to cirrhosis. These carriers are the object of screening procedures. Since they seem to have abnormal thymol turbidity, this test seems to be applicable as a screening procedure [3231]. Blood donors with a history of hepatitis or unexplained recent disease, with abnormal physical findings in connection with the liver, and with bilirubinuria, urobilinogenuria, elevated thymol turbidity, and increased cephalin flocculation, should also be excluded [2409].

Incubation Period. The incubation period of A virus is usually 15 to 40 days and for B virus, 40 to 160 days [1420, 2409] (Fig. 137). The prolonged incubation period of B virus has been

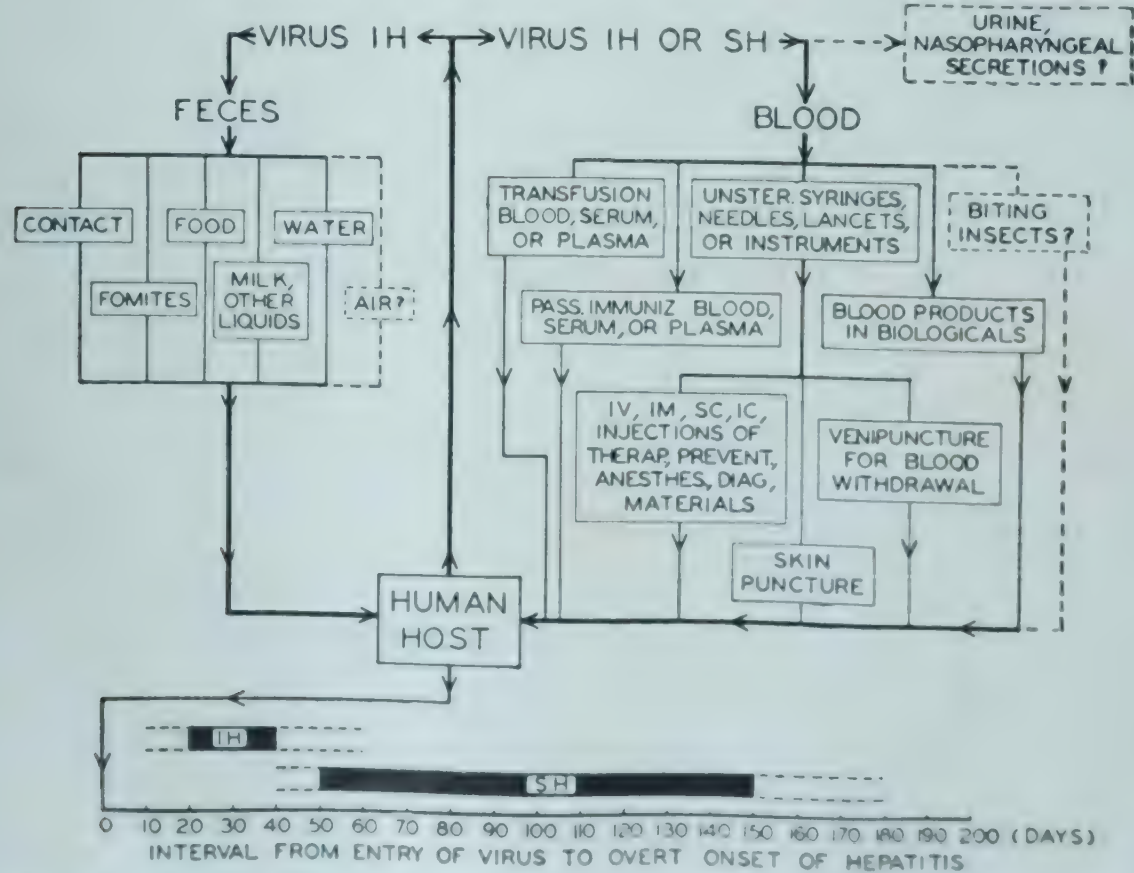


FIG. 137 Modes of transmission and incubation periods of infectious hepatitis. (Neefe, J R.: *New England J. Med.* 240:445, 1949.)

ascribed to inoculation of the virus together with serum antibodies [126]; but this is not supported experimentally by parenteral administration of convalescent serum [1420]. Parenteral transmission of A virus accounts for syringe or transfusion hepatitis with short incubation periods. The length of the incubation period also reflects the amount of the inoculum or an alteration of the virus. The incubation period of orally transmitted A virus hepatitis is prolonged if the amount of virus is reduced by various sterilizing procedures or by dilution of the infecting agent [3230].

Immunity. A VIRUS. Epidemiologic data suggest a relative immunity to reinfection after A virus infection [469, 1011], which has been confirmed in human volunteers [1420, 2413]. The immunity does not seem to be absolute and may be lost again, as indicated by negative skin tests. Second attacks, not in the form of relapses, are rare [2409] and do not occur in excess of 5 per cent of all cases [1136]. If they occur, they are probably the result of a heavy exposure. A virus hepatitis is

relatively uncommon in elderly people, suggesting acquired immunity during life. This supports the concept of a single strain of A virus.

B VIRUS. The immunity produced after exposure to serum hepatitis is rather short-lived [827, 2409, 2413]; it may be up to 1 year [2409]. Neutralizing antibodies have not been found [3232], and skin tests are negative. Furthermore, the incidence of B virus hepatitis does not change with increasing age, also suggesting the existence of several strains. Cross immunity between A and B viruses does not exist, as demonstrated in human volunteers [1420, 2413]. Clinical observations suggest that a preceding B virus infection even increases susceptibility to A virus hepatitis [1136].

EFFECT OF GAMMA GLOBULIN. The immunologic observations are also reflected in the protection afforded by gamma globulin. This serum fraction has proved effective in A infections in institutional outbreaks and in larger epidemics, 0.01 ml per lb of body weight in a single injection being fully protective [165, 3233]. This protection lasts for at

Table 48 Observed Differences between Two Hepatitis Viruses

Observations	Virus IH (or A)	Virus SH (or B)
Observed in human volunteers with experimentally induced infections:		
Type of onset	Abrupt; febrile, often high fever; often one chill	Insidious; afebrile or temperature usually less than 100°F; rarely a chill
Abnormal hepatic test results . . .	Preceded by symptoms for several days	Often precede symptoms by several or many days
Thymol and colloidal gold responses	Usually abnormal	Frequently negative or only weakly positive
Usual incubation period (from virus entry to clinical onset)	2 to 6 weeks	1½ to 6 months
Route of inoculation	Clinical hepatitis follows either oral or parenteral entry after 2 to 6 weeks	Clinical hepatitis after parenteral but not after oral entry
Virus demonstrated in	Blood and feces	Blood only
Oral ingestion of known infectious serum	Clinical hepatitis	No hepatitis
Parenteral injection of infectious serum	Clinical hepatitis	Clinical hepatitis
Oral ingestion of feces suspensions	Clinical hepatitis	No hepatitis
Resistance to reinfection after		
Virus IH	Present	Absent
Virus SH	Absent	Present (up to 1 year)
Based on epidemiologic studies:		
Incidence after 30 years of age . . .	Sharp decrease	No apparent decrease
Prevention by prophylactic injections of gamma globulin (human ethanol precipitate)	Yes	No

Source: After J. R. Neefe, *Am.J.Med.* 16:710, 1954.

least 9 months despite continued exposure, suggesting that the passive immunity conferred by gamma globulin is replaced by an active immunity. This active immunity is better induced by relatively small doses of gamma globulin. The immunity conferred by larger doses appears to last less than 2 months in most instances [830]. Serum hepatitis is not prevented by pooled gamma globulin [827, 3233], but some prolongation of the incubation period and some degree of protection result. Gamma globulin from the blood of patients convalescing from serum hepatitis fails to exert any protection in volunteers inoculated with the same strain of B virus, and the virus is not neutralized in icterogenic serums [827]. This suggests the lack of production of sufficient antibodies in B virus infections.

DIFFERENCES BETWEEN A AND B VIRUSES. Immunologic differences appear well established (Table 48). Simultaneous infections with two viruses seem to occur. Recurrent A virus hepatitis may in reality be B virus hepatitis developing months after an attack of A virus hepatitis because of the longer period of incubation characteristic of the B virus [2099].

Epidemiology

Virus A, usually acquired by the oral route, is transmitted by fecal contamination of water or by food handlers. Apparently, a large portion of the population acquires A virus hepatitis in childhood, mostly in an anicteric form. This results in immunity encompassing almost the entire population over thirty years of age. Sporadic cases of A virus hepatitis are seen all over the world. After massive exposure, such as occurs in army installations, in troops entering endemic areas such as southern Germany, North Africa, and Korea, or in civilian institutions, epidemic outbreaks result.

Virus B hepatitis, requiring parenteral transmission either by purposeful administration of blood products or by accidental contamination of syringes, needles, or lancets, or through broken skin wounds, is apparently less common than A virus hepatitis. Since it fails to confer long-lasting immunity, it is not restricted to any age group. It is usually sporadic, but an increased incidence, resembling an epidemic, occurs either following mass administration of plasma or plasma products, such as vaccines, or after contamination of syringes in an institution (Fig. 137).

Epidemiology of A Virus Hepatitis. Historically, epidemics of viral hepatitis first became known in

military personnel. The two world wars have given much impetus to the study of this disease, which was formerly called "camp jaundice" or "jaundice of campaigns." In the Civil War, Franco-Prussian War, and Boer War large epidemics occurred. In World War I, epidemics were noted in the armies of almost all the participating nations [1392, 2083]. In World War II, it became one of the major military medical problems in all armies [2083, 3495]: in the Mediterranean theater [156, 469, 1136, 1392, 2118], in England [2102], in the Burma theater [3235], in the German Army [793], and in the occupation troops in Germany [2541]. In some campaigns, the number of cases exceeded the number of battle casualties [1392]. About 250,000 cases occurred in American military personnel [3118], over 30,000 of which developed in North Africa alone [1392]. The military experiences emphasize that close contact among the troops is an important aspect in the transmission [2118, 3495]. In the occupation troops in Germany [2541], contact with a civilian population endemically infected with virus A was important. In the United States Navy, cases occurred all over the world, with no increased incidence in battle areas [405]. These military experiences implicated food handlers and contaminated water supplies as the most important sources of epidemics [1706].

Civilian epidemics, including epidemics of abortive cases, have also been known for many years.

With the introduction of transmission to human volunteers and skin testing, tracing the epidemiology of the disease more accurately became possible, whereas without these procedures, even in recent epidemics detailed epidemiologic information could not be obtained [685]. In several epidemics, water was found to be the probable source of infection [983, 1595, 2417, 3233], although personal contact has still not been completely excluded [165, 2102], especially in epidemics in school children [1807]. Fecal-oral spread through direct contact was demonstrated during an epidemic of hepatitis in nurses in an orphanage; the spread was halted after aseptic nursing technique was introduced in the management of nonicteric carriers [490]. The carriers themselves were probably also infected through the fecal-oral route [218].

The increase in incidence of viral hepatitis in the fall and winter [1392, 2409] is not explained. Transmission by blood-sucking insects has been also suggested [469, 2101], but no clear-cut an-

swer is yet available from studies on volunteers [2409].

PROPHYLAXIS. The prophylaxis of a disease in which the transmission is as incompletely understood as that of infectious hepatitis is difficult. The importance of the fecal-oral route suggests that hygienic measures directed against fecal contamination are the most effective procedure. Partial isolation of patients is recommended during the first week of their illness, with disinfection of feces, urine, and vomitus. When the patient is released, his room and utensils should be sterilized. Nurses taking care of children with hepatitis should use sterile technique in handling stools. Drinking water in endemic areas and during epidemics must be treated. Chlorination is not effective; more extensive methods are needed [2411].

During epidemics, gamma globulin can be used to confer immunity, but mass prophylaxis should be reserved (except in severe local epidemics [405]) for the intimate family and nursing contacts [165, 412] and for troops in highly endemic areas. In preventing spread of the disease in family groups, administration of 0.01 ml gamma globulin per pound of body weight suffices [1566].

Epidemiology of B Virus Hepatitis. Serum hepatitis virus is probably an old infectious agent of the human race which now lives in almost perfect symbiosis with man. It has apparently been transmitted from one generation to the next, transplantally, without producing clinical symptoms and without conferring immunity [3231]. These normal host relations have recently been disturbed by man introducing the virus into new hosts via parenteral routes.

The peculiar epidemiology of serum, or B, virus hepatitis also applies to A virus hepatitis, except for the shorter incubation period of the latter, if it is transmitted by a parenteral route. Five principal types of parenteral transmission occur: (1) transfusion of blood, plasma, serum, or blood fractions; (2) administration of vaccines containing human plasma or serum; (3) contamination of syringes and needles used in parenteral injections, venipunctures, or skin punctures for blood counts; (4) laboratory or occupational infections through handling of blood specimens or cleaning of contaminated needles; (5) contamination through open lesions of skin and mucous membranes. Many examples for each of the types of transmission have been recorded, many of them long before the concept of viral hepatitis existed; thus, some cases are understood only in retrospect.

Since most instances of serum hepatitis result either from medication or from blood examination, the opportunity for outbreaks has mainly occurred in the last 70 years. The first recorded outbreak resulted from smallpox vaccinations in 1885 [1420].

ANTISYPHILITIC-TREATMENT JAUNDICE. The frequent occurrence of hepatitis during antisyphilitic treatment suggested that an infectious agent was responsible. In retrospect, it appears strange that the incidence varied as much as it did, that no correlation between hepatitis and dosage or type of therapy was demonstrated, and that continued treatment despite the development of jaundice did not aggravate the hepatitis. Nevertheless, the true nature of this disease was not recognized, and outbreaks in venereal disease clinics were associated with other causes, even with gonorrhea, in which jaundice appeared only if injection treatments were used. Now, all these instances are thought to be the result of transmission of the virus via syringes, particularly since the histologic appearance of the liver is identical with that in infectious hepatitis [785, 2801]. As little as 0.001 ml whole blood is sufficient to transmit the disease [2409], and many of the commonly used sterilization procedures do not inactivate the virus [1420, 2409]. The importance of the disease in venereology has been statistically analyzed, and disappearance of the disease after introduction of proper sterilization techniques has been noted [2216].

SYRINGE OR INSTRUMENT-TRANSMITTED HEPATITIS. Outbreaks of hepatitis occur with multiple-dose-per-syringe techniques used for inoculations [492], for insulin administration [3047], or by drug addicts [57, 87, 3180]. Instruments used in obtaining blood for hematologic examinations also transmit the hepatitis virus [2718] and may be even more important than transfusions themselves [2718]. Transmission by dentists [1376] and by tattooing [3095] has been reported. The occupational hazards for doctors and personnel working in laboratories and blood banks belong in the same group [1675, 1873, 2173, 2915, 3365].

YELLOW FEVER VACCINE JAUNDICE. The development of about 25,000 cases of hepatitis in United States Army personnel after administration of yellow fever vaccine was the largest single outbreak of B virus hepatitis. It presented an urgent military problem during mobilization [1011, 1392, 2890]. Jaundice developed weeks or months after the vaccination and was not associated with the char-

acteristic signs of yellow fever. The disease was transmitted to human volunteers [2477]. In the preparation of the vaccine human serum had been used as a neutralizing agent; its replacement by animal serum abolished the disease. The icterogenic factor in the original vaccine was then identified as B virus [1010, 2890]. A few instances of chronic liver disease and cirrhosis have been reported without an acute icteric phase 10 years after inoculation with known icterogenic lots of yellow fever vaccine [396].

CONVALESCENT-SERUM JAUNDICE. In England and subsequently in this country jaundice was reported in groups of persons, particularly children, who had received pooled measles convalescent serum or mumps convalescent serum [203, 2414]. Epidemiologic studies indicated the presence of an icterogenic agent in some of the batches of serum administered.

POSTTRANSFUSION HEPATITIS. Jaundice which develops several weeks or months after transfusion of blood or plasma is the best-known form of serum hepatitis. Since its first description early in World War II in England and in the United States [203], many instances and fatalities have been recorded in military personnel [2085] and in civilians [2787, 2852], including small infants [2937] and children treated with plasma [2819]. Any jaundice developing 1 to 8 months after a transfusion must be considered serum hepatitis until proved otherwise. Jaundice which develops several weeks after operations on the biliary tree should first be considered viral hepatitis, rather than the result of a surgical mishap.

Several statistical studies of the incidence of posttransfusion hepatitis have been reported. Single blood transfusions cause jaundice in 0.5 to 1.5 per cent of recipients [2409]. With varying numbers of transfusions, the incidence remained about 1.0 per cent [2173]. A much greater incidence is found with pooled plasma; the larger the pool, the more likely the development of jaundice [484, 2119]. Following transfusion with plasma from large pools of 1,000 to 5,000 units, jaundice develops in 4.5 to 12.2 per cent of cases [2306, 3163]. Plasma from medium-sized pools (100 units) produces jaundice in 3 to 4 per cent of cases [2099], while from small pools of 5 to 10 units jaundice develops in 1.5 per cent of cases [2409]. A very high incidence of jaundice has been seen following the combined use of blood and plasma [2894].

All these figures are subject to many questions.

Aside from the possibility that the associated blood counts are responsible and not the plasma or blood transfusion [2718], the status of the patient at the time of the transfusion influences the incidence, morbidity, and mortality. Common colds or allergies possibly predispose to the disease [2308]. Since patients receiving transfusions are ill and frequently malnourished and exhausted, the subsequently developing hepatitis is more severe and has a higher mortality rate. This accounts for the commonly heard statement that serum hepatitis has a greater mortality and morbidity than infectious hepatitis [2085, 2852].

Of the processed blood products, serum albumin is safe [1145], as is gamma globulin [827], but human thrombin [1961], fraction IV from postpartum plasma [1565], and the antihemophilic fraction [3232] have produced the disease. The incidence of contact transmission of B virus hepatitis through minor cuts can not be evaluated at present.

PROPHYLAXIS OF SERUM HEPATITIS. Human serums in vaccines should be replaced by animal serums whenever possible. Occupational infections in laboratory personnel are best prevented by the exercise of care in handling blood or material containing blood derivatives.

Posttransfusion Hepatitis. The incidence of posttransfusion hepatitis and hepatitis from administration of blood products can be reduced by several means [2409]: (1) selection for transfusion of material entailing the least risk, such as serum albumin, whole blood, or single plasma units; (2) exclusion of infected donors (see Screening for Carriers, under The Carrier State, earlier in this chapter); (3) inactivation of hepatitis virus in blood and blood products (see Resistance, under Etiology, earlier in this chapter). Ultraviolet radiation reduces the incidence and morbidity but does not abolish the danger. Prolonged storage of liquid plasma or serum at room temperature or addition of beta-propiolactone decreases the incidence of hepatitis (see Resistance, above). Gamma globulin, either added to the blood or given simultaneously with it, fails to protect the patient adequately [827].

Syringe Hepatitis. Prevention of serum hepatitis transmitted by syringes and needles requires two steps: (1) the avoidance of multiple-injection-per-syringe techniques and of use of the same syringe for venipunctures on different patients without resterilization; (2) adequate cleansing and sterilization of instruments such as lancets after each

use. All instruments should be washed immediately after use to prevent organic material from coagulating or drying on various surfaces, interfering with subsequent sterilization. Immersion in alcohol or other antiseptic treatment and brief immersion in boiling water, both adequate for inactivating bacteria, do not inactivate the virus. Although largely abandoned, the multiple-injection-per-syringe technique is still employed by

many allergists for skin testing. For most purposes, autoclaving for 20 minutes at 15 lb pressure at 121°C is recommended [2409]. Boiling in water for at least 10 minutes is probably effective, as is dry heat at 180°C for 1 hour. Pins, phonograph needles, and wooden or glass splinters have been suggested to replace lancets for blood counts. Permanent lancets can be sterilized by flaming after washing in cold water.

HEPATIC INJURY FROM INFECTIOUS AGENTS: ACUTE VIRAL HEPATITIS

CLINICAL MANIFESTATIONS

Acute viral hepatitis occurs in four major clinical forms: (1) acute icteric hepatitis, previously called catarrhal jaundice, the most commonly recognized variety; (2) anicteric hepatitis, the incidence of which is not known because of difficulty in diagnosis; (3) hepatitis with severe hepatic failure, previously called "acute liver atrophy"; (4) cholangiolitic hepatitis causing intrahepatic cholestasis (Table 49). The first two forms do not differ from each other in laboratory findings or morphologic features, except that in the first there is bilirubinemia and bile pigment accumulation in the liver. In both, spotty necrosis of hepatic cells is the basic pathologic process, while acute hepatic

failure is usually reflected in massive necrosis. The viral etiology of acute icteric hepatitis with spotty necrosis is indicated by epidemiologic evidence and by the transmission of the virus in serum and stools to human volunteers. Epidemiologic observations suggest that the massive necrotic form with hepatic failure is also caused by the same virus; such cases appear in epidemics of acute icteric hepatitis [2083, 2085]. This conclusion is supported by individual incidents, such as the case of a pathologist who died from this disease 27 days after he cut his finger while performing a necropsy on a similar case [2308]. The evidence for the viral etiology of cholangiolitic hepatitis is, at best, circumstantial because of the sporadic appearance of such cases with possible exposures

Table 49 Clinical, Laboratory, and Structural Correlations in Viral Hepatitis

<i>Clinical stage</i>	<i>Laboratory findings</i>	<i>Structural alterations</i>
Anicteric hepatitis.....	Moderate hepatocellular degeneration, no jaundice	Spotty necrosis
Acute icteric hepatitis (catarrhal jaundice)	Hepatocellular degeneration, mild cholestasis, mild mesenchymal reaction	Spotty necrosis
Acute hepatic failure (acute atrophy)	Severe hepatocellular degeneration (low cholesterol and esters, low prothrombin, aminoaciduria)	Massive necrosis
Cholangiolitic hepatitis.....	Cholestasis	Periductular inflammation
Posthepatic neurasthenic dyspepsia	Nothing or variable mild hepatocellular degeneration or mesenchymal reaction	Normal liver or portal inflammation or nonspecific reactive hepatitis
Recurrent hepatitis.....	Hepatocellular degeneration, mild cholestasis, mild mesenchymal reaction	Spotty necrosis
Subacute hepatitis.....	Hepatocellular degeneration, variable cholestasis, severe mesenchymal reaction	Portal fibrosis with inflammation, variable hepatic-cell degeneration (postnecrotic scarring)
Cirrhosis (depending on stage and type)	Hepatocellular degeneration, cholestasis, severe mesenchymal reaction	Cirrhosis (mostly postnecrotic or septal, rarely cholangiolitic)

to the etiologic agent of viral hepatitis. In view of the fact that the viral etiology is rarely proved in the individual patient, separation from toxic hepatitis, especially allergic drug reactions (see Allergic Cholangiolitis, Chap. 41), is difficult if not impossible.

Clinical Differences between Virus A and B Hepatitis. Differences between infectious hepatitis and serum hepatitis can not be detected pathologically [125, 786, 1872, 2083, 2085, 2189]. Clinically, the differentiation between A and B hepatitis is extremely difficult, but experiments on human volunteers [2409, 2413] and observations in epidemics [1532] showed some differences (Table 48). The onset of virus A hepatitis is abrupt. Constitutional symptoms with fever are often severe in the beginning, and the laboratory findings of hepatic injury follow the clinical onset of the disease. In B virus hepatitis this condition is reversed. Sporadic instances of viral hepatitis without obvious exposure to B virus follow a course similar to that of serum hepatitis, which can be explained by infection during injections or examinations [50, 2409].

Acute Icteric Hepatitis. **PRODROMAL PERIOD.** A prodromal, preicteric stage precedes the icteric phase. The preicteric period, chiefly studied in military personnel, is missing in 20 per cent of the cases [156, 2897]. It is manifested by acute influenza-like upper respiratory symptoms, usually combined with gastrointestinal symptoms. The most predominant symptom is anorexia (in 65 per cent of cases) often associated with nausea (in 61 per cent) [2897]. Distaste for cigarettes is noted early [1940]. Vomiting, malaise, fatigue, pruritus, abdominal discomfort especially in the right upper quadrant, and abdominal distention also occur. Headaches, photophobia, and pain in the eyes have been noted. Fever, sometimes even with chills, is common [156, 2897, 3721]. The rise in temperature is often missed in clinical practice because the patients are not seen at this stage, but it has been demonstrated regularly in volunteers inoculated with A virus. The fever appears 1 or 2 days before the onset of jaundice and lasts until jaundice appears, or a short episode of fever may precede the appearance of jaundice by 10 days [1420]. The liver is not enlarged but it is tender, especially in the gallbladder region [156, 2897, 3721]. Lymphadenopathy, particularly in the posterior cervical region, has been reported [156]. Many other manifestations occur in isolated cases [1420, 3721]. Some of these are allergic in nature,

and the prodromal influenza-like symptoms may actually be allergic manifestations or may result from a predisposing common cold [2308].

PERIOD OF JAUNDICE. At the onset of jaundice, the gastrointestinal symptoms become aggravated, with nausea in the foreground, but they disappear at the height of the disease. In the early stages the patient is listless, anorectic, and very tired. The severity of symptoms varies greatly. Headache and itching are seen in about 20 per cent of cases. Fever is absent in the majority of cases. The jaundice gradually increases for about a week, and a few patients develop hepatic insufficiency, ushered in by central nervous system manifestations. Various other symptoms have been observed during this time, such as arthralgia, weight loss, urticaria, herpes, diarrhea, and dyspnea [156, 1383, 1420, 1505, 2897, 3721]. The liver enlarges slightly and is very tender, especially in the area of the gallbladder. Percussion of the right lower ribs elicits pain. The spleen is palpable in about half the cases and lymphadenopathy is common, especially in the posterior cervical region. Bradycardia, hypotension, spider angiomas, and palmar erythema are sometimes noted [1505]. Inflammation of the stomach and duodenum can be detected radiologically and gastroscopically [1426, 2072, 2544].

The duration of jaundice varies from a few days to several weeks and in rare instances is as much as several years. It lasts 1 to 3 weeks on the average in adults. In 15 to 20 per cent of cases, exacerbations occur during the course of the disease [156, 2409]. In the convalescent period a tendency for obesity may develop [631].

Anicteric Hepatitis. Some patients have a clinical course identical with that of acute icteric hepatitis, except that jaundice never appears [156]. The serum-bilirubin level remains normal or slightly elevated—up to 2.0 mg per 100 ml, i.e., subicteric hepatitis [2189]. Anicteric hepatitis seems to run a milder and possibly a shorter course than the icteric form. The diagnosis is difficult. Most instances are detected in military personnel during epidemics, when they may account for 70 per cent of instances of hepatitis [541, 2409]. Tenderness and enlargement of the liver with symptoms and laboratory findings identical to those of icteric hepatitis of a moderate degree were even encountered sporadically in civilians [767, 813, 3723]. In young children, sometimes the only symptom is diarrhea. The virus persists in the stools for periods of up to 5 years [218]. Awareness of the condition is important, since the

sequelae, including cirrhosis [396], resemble those of the icteric form.

Hepatitis with Hepatic Failure. In a few patients severe hepatic failure develops, preceded by central nervous system manifestations, and progresses to hepatic coma. Such a development is usually associated with a severe hemorrhagic diathesis. Frequently death follows the appearance of these ominous symptoms. The prodromal period is similar to that of the acute icteric form, with gastrointestinal and acute infectious symptoms. The fever also subsides with the appearance of jaundice. No relation exists between the intensity or duration of manifestations in the preicteric period and the final outcome. Indeed, acute hepatic failure and death occur even before jaundice appears or within the first 10 days of jaundice, i.e., fulminant hepatitis [2085], or later as delayed hepatic failure [2083]. Most fatalities result from serum hepatitis in military personnel [2085, 3651], civilians [3306], and even children [2937, 3652], but whether this represents a basic difference between the A and B viruses or increased susceptibility because of an accompanying disease is questionable.

The liver is initially enlarged but often shrinks, and the spleen is palpable in one-fourth of the cases. Ascites is found in many fatal cases, especially if death occurs after 10 days [2085].

Cholangiolitic Hepatitis. Since only some instances of intrahepatic cholestasis, or cholangiolitis, result from the hepatitis virus [3510] reference is made to the discussion elsewhere (see Intrahepatic Cholestasis, Chap. 24; also Cholangiolitis and Pericholangiolitis, Chap. 46).

Factors Modifying the Clinical Course of Hepatitis. Many factors in addition to immunity influence the clinical course of viral hepatitis and account for the variation in susceptibility of persons equally exposed.

MALNUTRITION. Malnutrition increases the severity of the disease among famine victims in India [1497]. The increased severity of serum hepatitis in drug addicts is also probably a result of associated malnutrition. Moderate hypoproteinemia has no effect on the severity of the disease [3040].

ALCOHOLISM. Chronic alcoholism or acute alcohol excess appears to increase the severity of the disease [156, 1383], although this has been denied [1128].

OTHER DISEASES. The aggravating effect of other diseases, including enteric and respiratory infections [1383, 1430], is well known [1430, 3336].

The greater morbidity and mortality of serum hepatitis are probably the result of the disease or injury for which blood or plasma was given [2085, 3175].

CHILDHOOD. In children hepatitis is usually less severe, and anicteric forms are common [218], although fulminant hepatitis, especially serum hepatitis, has been described in very young infants [2019], with a high mortality rate, especially when caused by virus B [1144, 2330]. Transmission of hepatitis in utero has been claimed [3234], and the passage of the virus through the placental barrier has been demonstrated. Congenital cirrhosis has been found in children whose mothers had jaundice during pregnancy [208, 3231]. The viral etiology of a peculiar type of hepatitis seen in the neonatal period, although strongly suggested [679, 784], has not been proved; therefore neonatal hepatitis with giant cells is described under hepatitis of unknown etiology (see Giant Cell Hepatitis, Chap. 46).

PREGNANCY. Most observers do not consider the incidence of true viral hepatitis to be increased in pregnancy over that in the general population [2838, 3729]. Some feel that the prognosis is worse in pregnancy [1144], especially in the third trimester and the post-partum period [2055], while others state that the prognosis is unaltered [1588, 2838]. No congenital anomalies follow hepatitis during pregnancy, nor do spontaneous abortions or stillbirths appear to be significant hazards. Severe massive necrotic hepatitis seems to be unfavorably influenced by pregnancy, and the mortality rate is increased. This form has only recently been separated from the toxemias of pregnancy [3033]. Infectious hepatitis during pregnancy is not considered an indication for therapeutic abortion [2838, 3334, 3729], except in severe cases [2231, 2448].

Outcome and Sequelae. Few patients with acute hepatitis die in the acute stage. Most of them recover completely, but about 20 per cent of adults seem to enter a subacute stage (see Types of Protracted Hepatitis, Chap. 44). A few develop biliary dyskinesia with cholecystitis [1677] after the acute stage. Alteration of the hepatic excretion of bilirubin sometimes follows the acute stage. This has been interpreted by some as hemolytic jaundice, but it seems to be retention jaundice and is identical with familial nonhemolytic jaundice, which may be only a convalescent stage of abortive viral hepatitis [1576] (see Nonhemolytic Retention Jaundice, under Proposed Classification of Jaundice, Chap. 21). Neurologic manifestations of

the Guillain-Barré type [3720] or generalized or focal cerebral signs sometimes follow hepatitis [2788].

MORTALITY. In general, death in the acute stage of viral hepatitis is rare, the incidence depending on accompanying factors and on the virulence of the virus. The death rate is not higher than 0.4 per cent [2083] and is usually about 0.2 per cent [2409]. However, in selected groups, very high mortality figures have been quoted, particularly in homologous serum hepatitis [3175, 3235] and in some epidemics, such as those in Scandinavia [231].

LABORATORY FINDINGS

Prodromal Period. The prodromal period has been best studied in human volunteers [1420, 2416]. The first abnormal result of the hepatic tests is Bromsulphalein retention, soon followed by bilirubinuria before the serum bilirubin increases. Subsequently, the results of cephalin flocculation become abnormal simultaneously with an increase in prompt-reacting serum bilirubin. The total serum bilirubin rises and the urinary urobilinogen increases just before jaundice appears. This indicates that for screening in the preicteric period, Bromsulphalein retention, urinary bilirubin and urobilinogen excretion, and the cephalin-flocculation tests are the most valuable procedures [156, 1420, 2416, 3281].

Acute Icteric Period. In the fully developed stage of icteric hepatitis, the results of almost all tests indicating hepatic-cell degeneration are abnormal [156, 1420, 1505, 2412, 2632, 2897]. The percentage of abnormal results depends upon the severity of the disease rather than on the duration of jaundice [2897]. The results of the thymol-turbidity and cephalin-flocculation tests are most frequently abnormal. Bilirubinuria is present, and serum albumin, cholinesterase, mucoproteins [1274], and prothrombin determined by the two-stage method [2207] are reduced. Gamma globulin and zinc sulfate turbidity are moderately increased, and beta globulins are slightly increased [2627, 2636, 2766]. Serum-alkaline phosphatase activity is increased but infrequently above 10 Bodansky units, except in children [2709]. The cholesterol ester ratio is usually depressed early, whereas total serum cholesterol is reduced only in severely ill patients [37, 1129]. Exceptionally, serum-alkaline phosphatase activity and total serum cholesterol are high as an indication of a

cholestatic component. The serum phospholipids are decreased in severe hepatitis but elevated in milder forms [37, 1505]. Plasma vitamin A is also reduced in severe hepatitis [1505, 2641].

The urinary urobilinogen excretion exhibits a biphasic pattern, in that early in the icteric stage the excretion is increased; it drops at the height of the disease, disappearing almost completely for a variable period lasting from 1 or 2 days to several weeks in exceptional instances [3183, 3185]. Simultaneously urobilinogen disappears from the stool (see Urobilinogen in Urine, Chap. 36). Cylindruria is common, while albuminuria and some impairment of concentrating ability of the kidney are noted occasionally [982].

The sedimentation rate is normal in early stages of the disease but rises later, dropping again during convalescence [1505, 2788]. The red blood count and hemoglobin concentration are not significantly altered, but the red cells appear macrocytic, hypochromic, and flattened (target cells) [3630]. These cells appear to have a decreased fragility [330], although this has not been confirmed [1420]. Serum-iron levels are often very high [581], and intravenously administered radioactive iron disappears faster than normal, although excretion of iron is not increased [2576]. Leukopenia (lymphopenia and neutropenia) occurs simultaneously with the preicteric febrile reaction, followed by a period of relative lymphocytosis [1427] and monocytosis [156, 1505]. The lymphocytes are atypical in many instances [156, 3702, 3721]. Some are the "stress" lymphocytes found in many infectious diseases, such as tuberculosis, but occasionally the blood smear raises the suspicion of infectious mononucleosis (see Hepatitis Caused by Infectious Mononucleosis, Chap. 45), and plasma cells appear in the circulating blood, apparently from the spleen [2323].

Serologic tests for syphilis are positive in about 2 per cent of the cases [1425]. Various antibodies have been described, but they are not used diagnostically [874, 1420, 2479].

In the defervescent stage, water retention, which was present in the acute stage, ceases, and diuresis marks the beginning of convalescence [1893]. Bilirubinuria disappears, while the serum bilirubin is still elevated [2416]. The urinary urobilinogen increases at this time. Somewhat later the cephalin flocculation returns to normal, while the thymol turbidity remains elevated and in some instances even rises [1427, 1879]. The total bilirubin returns to normal in 3 to 6 weeks. The

prompt-reacting fraction becomes normal when liver damage subsides [2906].

Anicteric Hepatitis. In anicteric hepatitis, the same laboratory findings are obtained except for the serum-bilirubin elevation. The percentage of abnormal results of the thymol-turbidity and cephalin-flocculation tests is high, since abnormal results of these tests are generally required to make the diagnosis [767, 813, 3723]. Urinary urobilinogen excretion and Bromsulphalein retention are common.

Acute Hepatic Failure. The laboratory findings in fulminant hepatitis are those seen in any type of catastrophic hepatic failure with hepatic coma. The bilirubin level is very high except in rare instances of sudden death in the prodromal period. The serum-cholesterol level and cholesterol esters are very low, and aminoaciduria is present. The NPN is usually not elevated, in contrast to the situation in toxic hepatitis [2632], the alpha globulin disappears [2627], and the serum-mucoprotein level drops. The blood-sugar level may drop [2085]. Anemia, leukocytosis, and thrombocytopenia frequently develop [3591].

Cholangiolitic Hepatitis. The laboratory findings of severe jaundice, usually with increase in serum alkaline phosphatase and total cholesterol but with normal results of flocculation tests and normal serum proteins in the absence of mechanical biliary obstruction, occasionally occur in viral hepatitis. These instances of laboratory evidence of primary cholestasis are discussed later (see Cholangiolitis and Pericholangiolitis, Chap. 46).

Therapeutic Considerations

Patients with uncomplicated viral hepatitis usually recover spontaneously; therefore reliable information as to therapeutic results must be based on large statistical studies possible only in military installations [537] or in controlled studies in volunteers [1950].

Strenuous physical activity is harmful, but absolute bed rest after the acute symptoms have disappeared is no longer considered necessary, at least in young and fairly well-nourished patients. Unrestricted activity should be permitted only if the laboratory findings meet the criteria of healing

(see Acute Icteric Period, earlier in this chapter). Some isolation procedures are indicated, especially to avoid contamination with feces and blood.

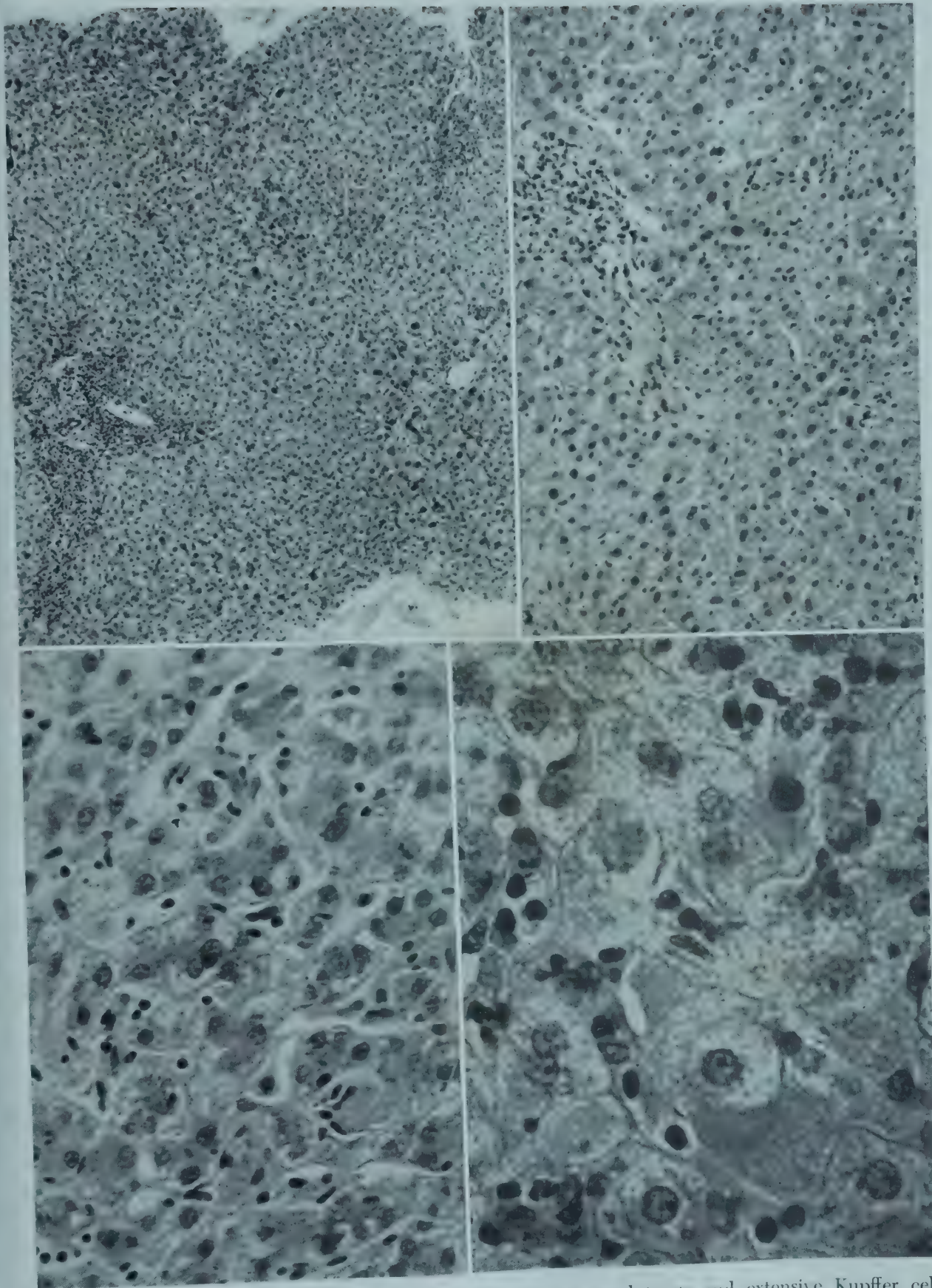
The diet should consist of at least 3000 cal with approximately 100 gm each of protein and fat. The prime requisite of the diet is appetite appeal to encourage eating, and no upper limit should be placed on the amount of food allowed in the acute stage. During convalescence some caloric restriction is indicated. In the period of severe anorexia and in critically ill patients, glucose infusions with vitamin supplements are recommended. Under these circumstances, administration of protein should be kept low, and protein hydrolysates are harmful [2588].

For the nausea and vomiting of the early stages, Benadryl, Pyribenzamine, and Dramamine can be tried. Chloral hydrate is preferable to barbiturates for sedation. Antibiotic treatment has been recommended, but its value is questionable. Gamma globulin or immune serum is of value only in prophylaxis. A beneficial effect has been reported from cortisone [959, 1428, 2771] and ACTH [10, 959, 2771, 2895]. Their use in uncomplicated hepatitis is unnecessary or even contraindicated because of undesirable side effects and the possibility of relapse if they are discontinued too early. They are apparently useful in small doses in relapses, chronic hepatitis, and cholangiolitic hepatitis. Massive doses are sometimes life-saving in impending coma, especially if combined with antibiotics [211].

STRUCTURAL ALTERATIONS

The pathology of viral hepatitis has been fully established only in recent years, although autopsy material of the fatal form has long been available. Histologic observations of nonfatal cases were limited to occasional instances in which patients died from other causes while suffering from hepatitis [3070] and in which biopsies were obtained during operations [1801, 2625]. Material obtained from soldiers killed in World War I while suffering from hepatitis has special historical interest [943]. The widely expanded use of liver biopsy made the systematic study of nonfatal hepatitis

FIG. 138 Biopsy specimens of acute viral hepatitis. H&E. *Upper left.* Low-power view at the height of the disease, revealing diffuse hepatocellular degeneration with centrilobular regeneration and focal accumulation of exudate cells in the parenchyma and in the portal tracts. Attempts at irregular regeneration in the lobular center ($\times 55$). *Upper right.* Early stage showing variations in shape and staining quality from one hepatic cell to another. Focal accumulation, chiefly of mononuclear cells



replacing necrotic hepatic cells. Some exudate cells also in portal tracts and extensive Kupffer cell mobilization ($\times 130$). *Lower left.* Great variation of neighboring hepatic cells, some showing ballooning of cytoplasm and some containing multiple nuclei; extensive mobilization of Kupffer cells, the cytoplasm of which becomes diffusely acidophilic ($\times 33$). *Lower right.* Irregular appearance of hepatic cells. Their cytoplasm is partly ballooned and partly homogeneously acidophilic. Many exudate cells are present, mostly mononuclear cells and a few segmented leukocytes ($\times 480$).

possible, and the findings of Scandinavian [2801], German [125, 1872], English [786, 3541], and American authors [1714, 2189, 3093] permit the description of the alterations in acute nonfatal hepatitis as well as its relation to the fatal form. The individual morphologic lesions in viral hepatitis are not specific, but the association of the various changes seldom occurs in other conditions [1677].

Morphologic Types of Acute Viral Hepatitis.

Three morphologic types of acute viral hepatitis can be distinguished: a form with spotty hepatic-cell necrosis, one with massive necrosis, and one with intrahepatic cholestasis, or "cholangiolitis," without necrosis. In the spotty necrotic form, death of scattered single hepatic cells produces an extremely polymorphous picture, which clinically corresponds to that seen in acute icteric or anicteric hepatitis. In the massive necrotic form, all or almost all of the cells of a lobule become necrotic. This development is associated with hepatic failure. Transitions between both forms exist, but morphologic evidence of such transitions is not often seen [1872]. Focal collapse in the chronic stage suggests that massive necrosis of isolated lobules occurs in cases without hepatic failure [1872]. In patients with massive necrosis, biopsies are rarely performed, because the prothrombin level is usually low; most studies are based on autopsy material. The contrary is true in the spotty necrotic form. Less convincing morphologic evidence is available for "cholangiolitis," in which demonstration of primary cholestasis is mainly based upon functional evidence.

Viral Hepatitis with Spotty Necrosis

The description of this form is based upon extensive reports in the literature [125, 786, 1390, 1714, 1872, 2189, 2897, 3093, 3541] and the study of patients at Cook County Hospital, many having multiple biopsies.

Preicteric Stage. Only few biopsies have been obtained in patients before the onset of jaundice [1576, 2189]. Therefore, little is known about the structure of the liver when the first clinical symptoms and fever appear. In the few biopsies obtained, the changes were identical to those in the icteric stage except for the absence of bile stasis.

Icteric Stage. In the first few days of jaundice the lobular parenchyma, mesenchyma, and the portal tracts are all diffusely involved, while signs of bile stasis, such as bile pigment accumulation in hepatic cells and Kupffer cells, are more severe in the center of the lobule (Fig. 139E).

PARENCHYMAL CHANGES. The liver lobules vary greatly in their appearance (Fig. 138, upper left). The hepatic-cell plates are irregularly bent without distortion of the lobular architecture as a whole [2189]. The glycogen content of the cells is significantly reduced only in severe damage [1601, 1872, 3541]. Extensive alterations of the hepatic cells, which vary from cell to cell in the same zone of the lobule, and focal regeneration of the intra-lobular parenchyma account for the polymorphous picture characteristic of the disease. It is best appreciated in methyl green pyronin stain, which brings out the great variations in cytoplasmic basophilia. Feathery degeneration near extravasated intralobular bile is found in severe jaundice.

Nuclear Variations. The nuclei vary in size and staining quality, some nuclei being hyperchromic and ballooned, while others are small [2801]. Exceptionally large acidophilic inclusion bodies are seen in the nuclei (Fig. 139B). Some of these changes are degenerative, others regenerative. The enlargement of the nuclei has been used for diagnostic purposes in smears of liver tissue [323].

Acidophilic Degeneration. The cytoplasmic basophilia is decreased to a variable degree [125].

Balloon Cells. Some hepatic cells become ballooned up to 50 μ m in maximal diameter (Fig. 138, lower left and right). Their rarefied cytoplasm appears as fine granules, which often are bile-stained and iron-containing [3093]. Their nuclei become hyperchromic, show regressive changes, and finally disappear, indicating necrosis of the cell. Most of these balloon cells are centrally located (Fig. 139C).

Necrosis. Normal-sized cells in the centrolobular zone become necrotic after extensive fine granulation of the cytoplasm (Fig. 138, lower left). Dense, ramified coagulated clumps, Mallory bodies, seen in other forms of necrosis are not observed [2187]. In severe instances necrosis involves several rows of hepatic cells around the central vein; cell remnants either are still visible or have disappeared (Fig. 139D).

Acidophilic Bodies. In some hepatic cells, circumscribed areas of the cytoplasm become densely and homogeneously acidophilic (Fig. 138, lower right). Eventually this involves the entire cytoplasm of the cell, which shrinks, while the nucleus becomes globular and pyknotic and finally disappears. Acidophilic bodies form which are extruded from the hepatic-cell plate [125, 1601, 1872, 2189, 3070, 3093] (Figs. 88A and B, 139A). They eventually are refractile globules with no

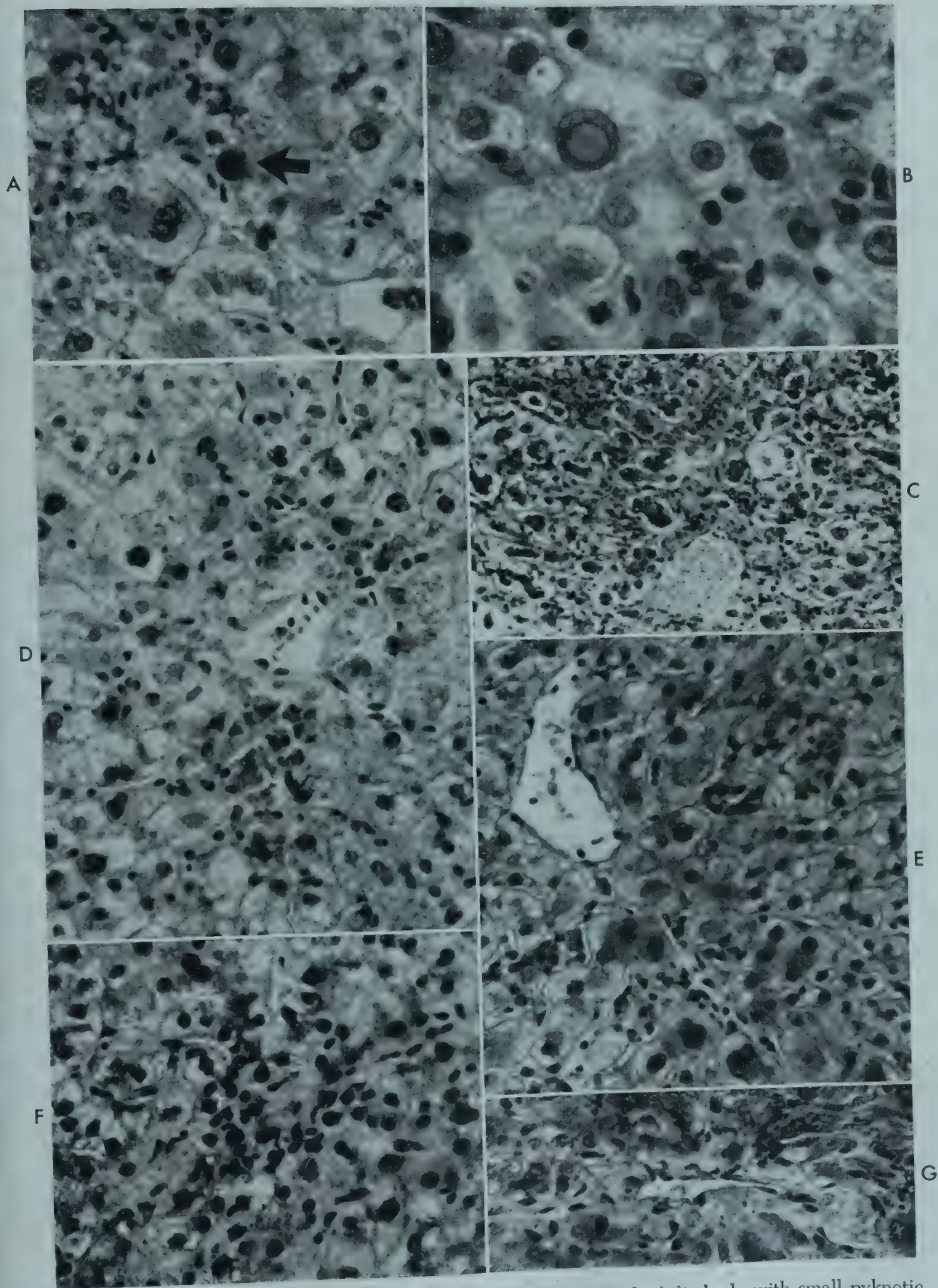


FIG. 139 Biopsy specimens of acute viral hepatitis. H&E. A. Acidophilic body with small pyknotic nucleus located in tissue space (arrow). The hepatic cells around it show hydropic swelling or acidophilic clumping of the cytoplasm. Extensive inflammatory reaction ($\times 295$). B. Acidophilic inclusion in nucleus. Severe hepatic-cell damage and spotty necrosis are seen ($\times 455$). C. Hydropic swelling of hepatic cells around central vein ($\times 115$). D. Bile imbibition of hepatic cells and Kupffer cells around central vein ($\times 220$). E. Centrolobular accentuation of hepatic-cell damage ($\times 230$). F. Accumulation of lymphocytes and histiocytes replacing necrotic hepatic cells (spotty necrosis) ($\times 230$). G. Swelling of wall of central vein ($\times 230$).

contact with surrounding cells, located in the tissue spaces or occasionally in Kupffer cells after phagocytosis. They resemble Councilman bodies found in the mid-zone of the lobule in yellow fever, but they do not contain the brown pigment characteristically seen in yellow fever. They are glycogen-free. Both the preceding homogeneous focal hyalinization of the hepatic cell and the fully developed acidophilic body differ in trichrome stains from the rest of the cytoplasm. The acidophilic bodies have been compared with inclusion bodies of other virus diseases [213]. Similar bodies occur in other human and canine viral disease and are also found in conditions not caused by viruses (see Acidophilic Bodies, under Cytoplasmic Coagulation, Chap. 22).

Bile Canaliculi Alterations. The cell membrane and the bile canaliculus wall stain poorly [1903]. Within the dilated bile canaliculi in the earlier stages nonpigmented protein casts are noted [1872].

Regeneration. Regeneration starts early. Around areas of necrosis, cells with dark cytoplasm are seen. Uninvolved cells, some of which contain clumps of glycogen, enlarge, and their nucleoli become prominent. Many mitotic figures are seen in the first week, whereas later multinucleated cells are common [1872, 2189, 2801, 3093]. In later stages irregularly distributed small giant cells are found [125, 1872, 2189, 3541] (Fig. 140D).

CHANGES IN INTRALOBULAR MESENCHYMA. The Kupffer cells proliferate and are mobilized, more in some areas than in others (Fig. 138, upper right and lower left). Mitosis is frequently seen; the cytoplasm is extremely basophilic and contains bile and iron-containing and iron-free pigments, in addition to cellular debris [1872]. The iron-free pigment gives the periodic acid and silver reactions and seems to be at least partly a polysaccharide. Kupffer cells also contain fatty material, which is in part acid-fast. Some of the pigment is a wear and tear lipochrome, probably derived from degenerating hepatic cells, since small amounts can be seen in hepatic cells. The increase in monocytes in the peripheral blood has been said to result from sequestration of proliferated Kupffer cells [125, 1872].

A few inflammatory cells, predominantly histiocytes and lymphocytes, accumulate around the necrotic hepatic cells. The remnants of the latter are usually invisible (Fig. 139F). Neutrophilic and eosinophilic leukocytes and plasma cells are occasionally intermixed.

The capillaries in the center of the lobule are dilated and congested with blood, and extravasation is noted around necrotic cells [1872]; but circulatory conditions are difficult to evaluate in biopsy specimens [125]. In places the capillary lumen appears obstructed by proliferated Kupffer cells, inflammatory cells, and protein debris. The reticulum fiber framework is intact and hardly ever reflects the parenchymal changes. Only when the central areas are completely denuded of hepatic cells does the reticulum fiber framework collapse, and the fibers then appear thickened. In such areas erythrocytes are found outside the sinusoids. Increase in collagen fibers is not noted. The walls of central veins are often diffusely and homogeneously thickened (Fig. 139G). Their collagen fibers are separated by amorphous protein, possibly from disintegrated hepatic cells, since it is usually seen when the hepatic cells in the center of the lobules are necrotic [3541].

CHANGES IN PORTAL TRACTS. Many inflammatory elements, mainly histiocytes and in later stages lymphocytes, with a variable sprinkling of plasma cells and eosinophilic and neutrophilic leukocytes, are seen in the portal tracts. They are not arranged around bile ducts but occasionally accumulate around lymphatic vessels (Fig. 140A). The histiocytes contain the same pigments as the Kupffer cells. Some of the fixed connective tissue cells appear proliferated and exhibit iron-free lipochrome and occasionally some iron-containing pigment. Slightly stained edema fluid separates the collagen fibers, while the dilated lymphatic vessels are filled with protein-rich material. Fibroblasts with spindle-shaped nuclei are seen only in late stages.

The number of ductules is increased, mainly on the periphery of the portal tracts and only much later within the parenchyma. The ductular epithelium is usually normal, and the lumen patent. Rarely it contains bile plugs. In later stages, the epithelial cells show mitoses, and exudate is seen in the lumen. Inflammatory cells then accumulate around the ductules, both periportally and intralobularly (Fig. 140B).

When the periphery of the portal tracts is heavily infiltrated and the ductules proliferate, the limiting plates are destroyed. As the inflammatory process extends into the parenchyma, the hepatic cells on the periphery of the lobule disappear, and the framework collapses [3541]. The intralobular bile ducts and the branches of the portal vein and hepatic artery are not involved.

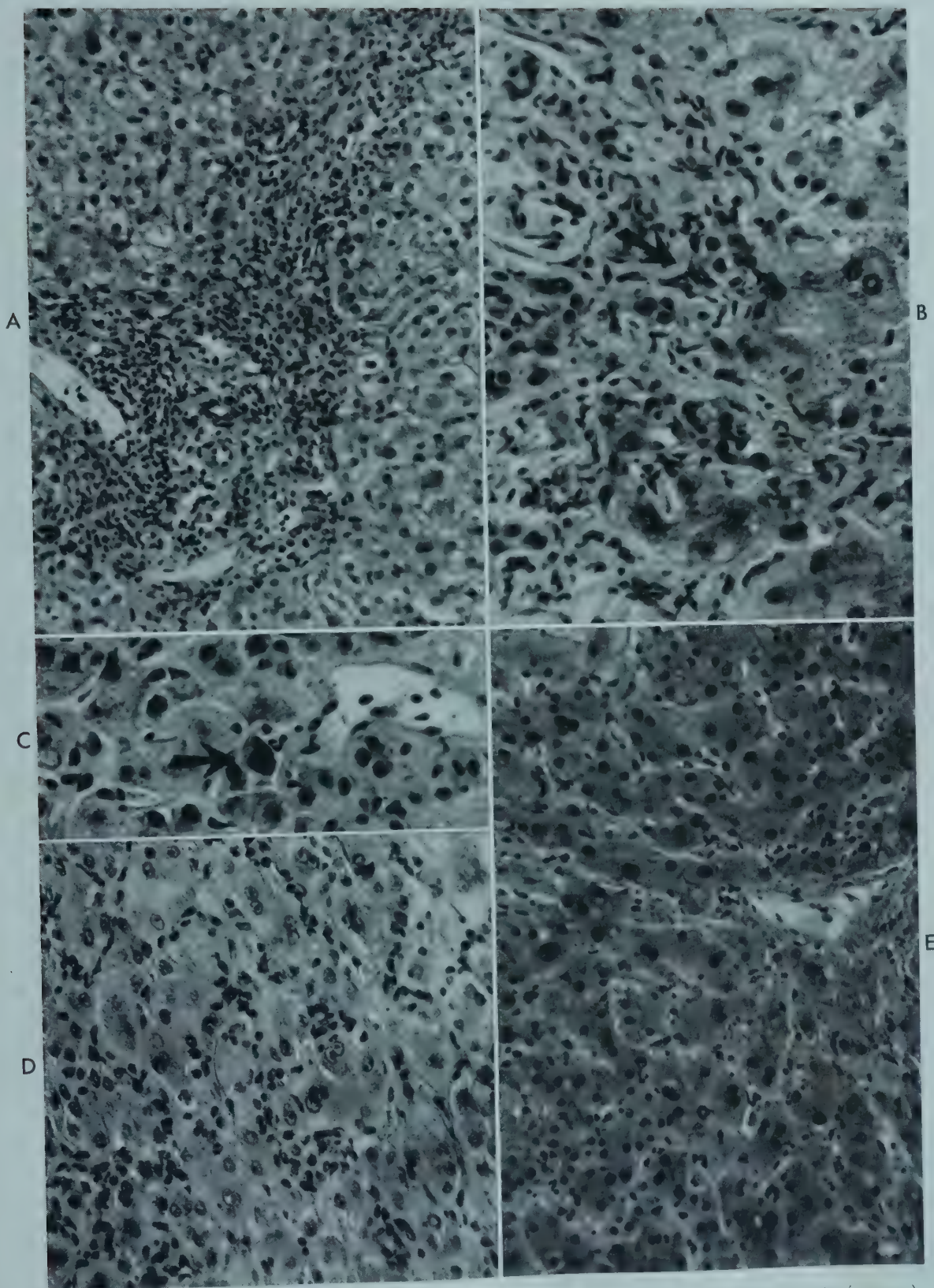


FIG. 140 Acute viral hepatitis. H&E. A. Portal inflammation and periportal necrosis ($\times 115$). B. Proliferation of ductules (arrow) surrounded by inflammatory exudate and injured hepatic cells ($\times 230$). C. Cast in dilated bile canaliculus in later stages (arrow) ($\times 275$). D. Intensive regeneration of hepatic cells with formation of multinucleated cells in late stages ($\times 275$). E. Autopsy specimen showing spotty necrotic viral hepatitis in left lobe, while right lobe shows massive necrosis. Note variations of size and shape of neighboring hepatic cells and spotty necrosis ($\times 130$).

Edema, in the sense of serous hepatitis, described as an important feature of the disease [125, 943, 3070], is not found in biopsy specimens.

Histologic Variations. The histologic changes vary in severity and in the various stages of the disease [125, 1872, 2189, 2801]. The intralobular and periportal mesenchymal reaction develops early, approximately from the first to the tenth day of jaundice, and exceeds the necrosis of the epithelial cells in intensity. Spotty necrosis is usually more severe on the periphery of the lobule [1872]. In the second 10 days, the degenerative changes of the parenchyma and the inflammatory changes become more conspicuous. The disarray of the hepatic-cell plates is at its height, and in the center of the lobule balloon cells and extensive necrosis appear in severe cases. Hepatic cells and Kupffer cells are bile-imbibed, but bile thrombi are not numerous.

In the defervescent stage, usually after 20 days, the necrotizing changes recede, especially those in the center of the lobule. Necrosis of single cells and the acidophilic bodies persist for a long time. Sometimes hepatic cells do not grow into areas of focal necrosis, and the framework collapses, leaving minute scars. Regeneration becomes very conspicuous. Bile plugs are seen frequently only in later stages [1782] (Fig. 140C). Reaction around bile extravasates may persist. The intralobular mesenchymal reaction becomes more localized and focal, while the periportal infiltration persists the longest (Fig. 141, all parts). Structural recovery is much faster in some patients than in others.

Spotty necrosis is not often seen in autopsy material of viral hepatitis. It may be found in livers with fatal massive or submassive necrosis, in the parts or lobes which are spared from massive necrosis. All the features of spotty necrosis are noted in these areas, except that the inflammatory reaction around the sites of necrosis is somewhat subdued (Fig. 140E).

Morphogenesis of the Spotty Necrotic Form of Hepatitis. The morphogenesis is in keeping with the hypothesis that the hepatitis virus is hepatotropic and causes degeneration of the hepatic-cell cytoplasm and probably also of the nucleus. This degeneration proceeds to granular or diffuse coagulation necrosis and possibly to autolysis. The hepatic cells disappear. The entire liver is involved, explaining the functional manifestations of diffuse hepatic-cell degeneration. The virus probably grows in different hepatic cells at dif-

ferent rates, and therefore hepatic-cell degeneration with the subsequent necrosis varies from cell to cell. This explains the lack of zonal involvement, since virus growth does not depend upon blood flow. A portal route of invasion is suggested by the early peripheral predominance of single-cell necrosis [1872].

The intralobular and periportal mesenchymal involvement has been interpreted as a reaction to the parenchymal changes [3541], as a primary lesion, "reticuloendotheliosis" [1529], or as a reaction to primary involvement of capillaries [3426]. That the hepatocellular involvement is primary and not secondary to mesenchymal change is well accepted. The extent of the mesenchymal changes, especially in the very early and late stages, suggests that the mesenchymal reaction is in part independent. It may result either from viral attack [125, 1872] or from an "allergic" reaction to viral products [478]. The high gamma globulin level in late stages in the absence of functional or morphologic evidence of hepatic-cell damage also indicates an independent mesenchymal reaction. The alteration of the ductules also found in later stages suggests that they too are attacked by the virus. In some instances ductular involvement may become more prominent, resulting in a severe cholestatic component, which is more reflected in laboratory findings in the acute stage than in histologic changes. In cholangiolitic hepatitis the cholestasis is the only significant lesion, and hepatic-cell degeneration is in the background (see Intrahepatic Cholestasis, Chap. 24; also Cholangiolitis and Pericholangiolitis, Chap. 46).

In addition to the diffuse single-cell necrosis attributed directly to the virus, central necrosis occurs, which is best explained by secondary effects upon the intralobular circulation. The Kupffer cell mobilization and inflammatory infiltration augmented by protein debris focally obstruct the blood flow. This does not account for all the changes, as has been claimed [125]; but it does explain ballooning of hepatic cells, which is a result of anoxia [55, 1172, 3361]. The balloon cells further compromise the intralobular circulation. These anoxic changes proceed to granular coagulation necrosis, predominantly in the center of the lobule. Coalescence of these central changes explains the centrolobular zonal necrosis resembling toxic hepatitis or congestive necrosis reported in earlier publications, especially in severe cases [125, 786, 2801, 3070]. These changes have not been found in other studies [2189]. The mes-

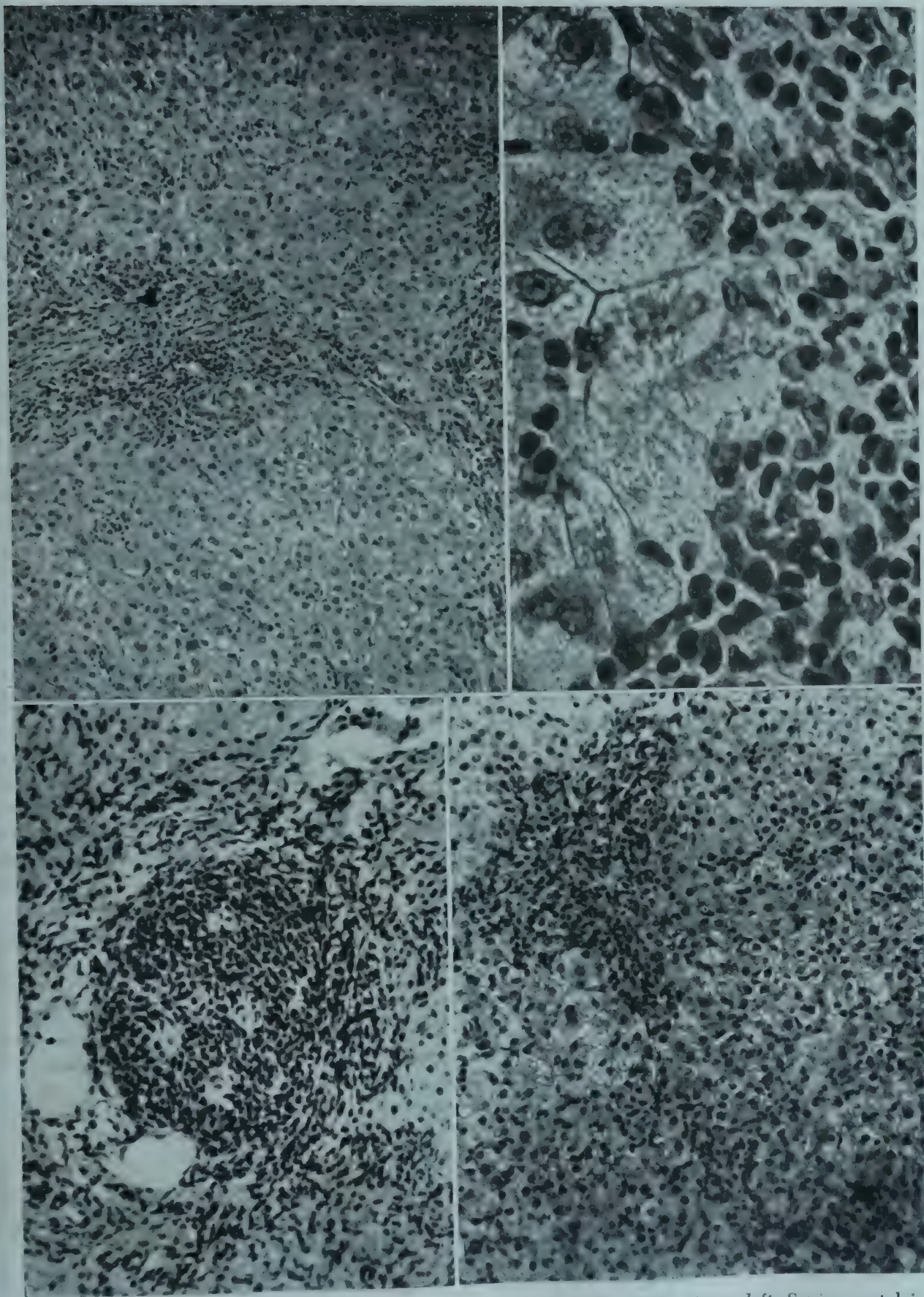


FIG. 141 Biopsy specimens of late stages of acute viral hepatitis, H&E. *Upper left.* Severe portal inflammation and intralobular accumulation of mesenchymal cells. The hepatic cells are almost normal ($\times 120$). *Upper right.* Border of a nodule composed of mesenchymal cells in nodular form in portal tract ($\times 190$). *Lower left.* Dense accumulation of mesenchymal cells collecting in a portal tract and extending as streaks or small nodules throughout the parenchyma ($\times 190$).

enchymal reaction also accounts for peripheral necrosis. The severe portal inflammation associated with ductular proliferation interferes with the nutrition of the cells on the periphery of the lobule.

Histologic Differential Diagnosis of Spotty Necrotic Form. The recognition of viral hepatitis in liver biopsy specimens is of clinical importance. The appearance is diagnostic only in fully developed stages, and the absence of characteristic features does not exclude viral hepatitis. Diagnostic criteria vary in significance.

1. Acidophilic, or "Councilman," bodies are almost pathognomonic.

2. Polymorphism of the hepatic-cell degeneration if severe is also almost pathognomonic, since it is rarely found in any other conditions except infectious mononucleosis. The polymorphism of the lobular parenchyma in florid cirrhosis (see *Florid Cirrhosis*, Chap. 51) is caused by membrane formation in addition to areas of focal necrosis, which contain predominantly segmented leukocytes.

3. Irregular regeneration with multinucleated giant cells irregularly arranged is almost as significant. It, too, is not seen in other conditions except infectious mononucleosis.

4. Severe intralobular mesenchymal reaction with accumulation of mononuclear cells around necrotic or missing hepatic cells is not often found in other conditions, but the presence of segmented leukocytes does not exclude viral hepatitis.

5. Large amounts of lipofuscin pigment in intralobular and portal mesenchymal cells intermixed with some iron-containing pigment are suggestive of viral hepatitis.

6. Hepatocellular fat in the acute stage speaks against viral hepatitis except in children or if it can be explained by other causes such as nutritional imbalance or antibiotic or steroid therapy. In convalescence, fatty metamorphosis occurs.

7. Changes in the portal tracts are not diagnostic.

In toxic hepatitis the involvement is zonal and polymorphism is absent, regeneration is zonal, mononuclear reaction is rare, segmented leukocytes predominate, and fat is commonly found. In extrahepatic biliary obstruction as well as in primary cholestasis, bile stasis is the most important change, with scattered feathery degeneration and bile imbibition of hepatic cells. The hepatic-cell plates are frequently narrow in the center of the lobules. The degree of bile stasis far exceeds the hepatocellular changes.

Anicteric Hepatitis. Histologic observations on anicteric hepatitis [125, 2189] are sparse. The findings are identical to those in the icteric form, except that the changes are usually not so severe. The major difference is the absence of bile stasis in the form of bile plugs and pigment deposition in Kupffer cells and hepatic cells.

Massive Necrosis

Many excellent gross and histologic descriptions of fatal epidemic hepatitis [2083, 2085, 3316, 3651] or "acute atrophy" are available [231, 1470]. Fulminant hepatitis can develop before jaundice appears or after it has been present for as long as 6 weeks. When it occurs late, it is either the result of sudden massive necrosis in spotty necrotic hepatitis with the preceding milder changes erased by the massive necrosis, or it is one form of hepatitis, particularly B virus disease, superimposed upon another. Regeneration following necrosis and collapse rapidly distorts the lobular architecture, producing a picture in a few weeks which does not differ significantly from that seen in chronic stages (see Chap. 44).

Macroscopic Appearance. Grossly, the liver is reduced in size, often weighing less than two-thirds its normal weight. The surface is homogeneously smooth or wrinkled. The anterior edge is sharp, and the color on surface and cut surface is usually mottled, varying between red and tan-gray (Fig. 142A). A yellow hue is rarely noted. In fulminant cases no difference is seen between the right and left lobes. Only in cases of more than 10 days' duration is the left lobe more involved [2085]. The architecture is sometimes exaggerated by distinct, often connected, red, depressed central areas contrasting with a tan-gray periphery. In other cases the lobular markings on the tan-gray cut surface can hardly be identified, and the cut surface looks like that of a spleen apparently after collapse of the lobular parenchyma. The difference depends mainly on the blood content. The differences are conspicuous throughout the liver in non-fulminant cases (Fig. 143A). The consistency of the organ is reduced. It can easily be bent and if placed on a board it flattens.

Histologic Alterations. FULMINANT FORM. In the first day or two, disruption of the hepatic-cell plates with swelling of individual cells, loss of basophilia, and, infrequently, formation of acidophilic bodies is seen [3651] (Fig. 142B). Then the hepatic cells disappear, occasionally leaving a small intact ring on the periphery of the lobule.

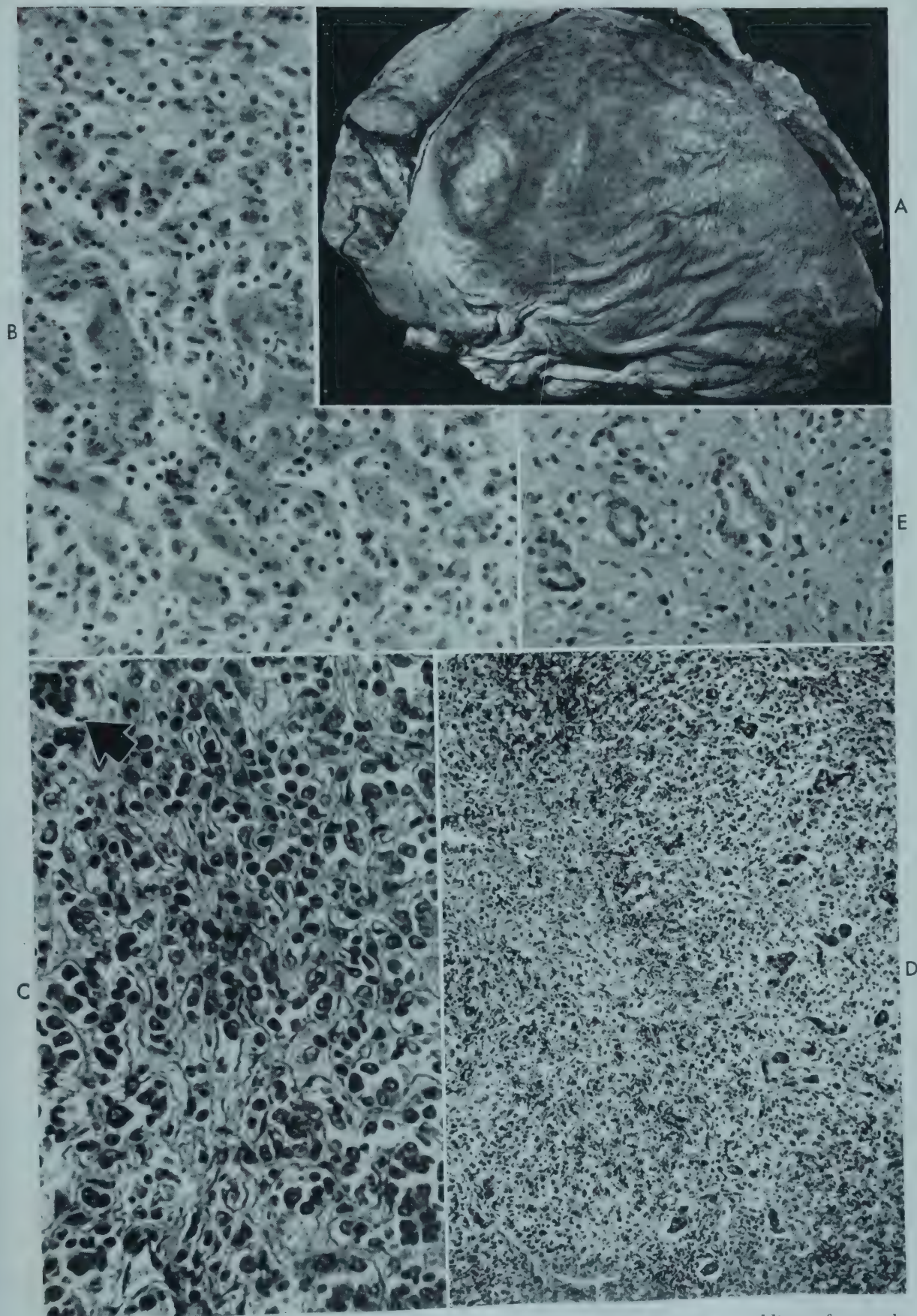


FIG. 142 A. Fatal fulminant viral hepatitis. Note softness of liver with wrinkling of capsule. B. Disintegration of hepatic cells, which contain much granular pigment and are surrounded by scavenger cells. H&E ($\times 270$). C. Scavenger cells completely replacing hepatic cells in the fulminant viral hepatitis. Note absence of hepatic cells and the presence of one ductule in the left upper corner. H&E ($\times 220$). D. Acute massive viral hepatitis. Hepatic cells have disappeared and are replaced by exudate cells. H&E ($\times 65$). E. Ductules appearing increased in number in collapsed stroma surrounded by scavenger cells.

The remaining cells become large, and their dense cytoplasm is fat-free. No signs of regeneration are seen in the fulminant form [2085]. Small anuclear hepatic-cell fragments remain but are poorly differentiated from the protein debris, except under the fluorescence microscope. The hepatic cells are replaced by proliferated and mobilized Kupffer cells intermixed with many histiocytes and lymphocytes and a few segmented leukocytes (Fig. 142C). The cytoplasm of the Kupffer cells is deeply basophilic and, like that of all histiocytic cells, contains much engulfed material, including iron-free brown pigment (see *Changes in Intralobular Mesenchyma*, earlier in this chapter) and fat droplets. The differentiation of the enlarged scavenger cells from hepatic cells may be difficult on low-power examination. Therefore on superficial examination hepatic cells may appear present when they have actually completely disappeared. The capillaries contain protein debris and erythrocytes, which are also found in the tissue spaces. The central veins appear thickened because of imbibition with protein, apparently derived from the disintegrated hepatic cells [2085], and occasionally also because of cellular infiltration (Fig. 143B). The framework is intact, although in the center some collapse and condensation are noted and the fibers are stained more easily than normal. The portal tracts are densely infiltrated with inflammatory cells (Fig. 142D). The perilobular and, to a lesser extent, the intralobular ductules proliferate, outlining the lobules, the architecture of which is preserved despite the disappearance of most of the hepatic cells (Fig. 142E). Bile casts are rare.

ACUTE, NONFULMINANT FORM. If death occurs later than 10 days after the onset of jaundice, the framework of at least some of the lobules has collapsed. Ghost lobules are found which are smaller than the original lobules, and the portal tracts are closer than normal. The periphery of the ghost lobule is demarcated by the periportal ductules, which appear to be increased in number (Fig. 143D). In other groups of nodules massive necrosis still seems to be progressing. Regeneration of the hepatic cells has started. They form multinucleated giant cells, and frequently they appear similar to ductular cells (Fig. 143C). In some parts of the liver, especially those which grossly show exaggeration of the architecture, the lobules are preserved but the hepatic cells are degenerating, and massive necrosis has not started.

Morphogenesis of Massive Necrosis. Massive necrosis is an exaggeration of spotty necrosis, with necrosis of single cells replaced by rapid necrosis of all or almost all the hepatic cells in a lobule. It is probably the result of a massive infection of most of the hepatic cells by the virus. This is supported by the occasional presence of acidophilic bodies, so frequently seen in the spotty necrotic form. In addition, the conspicuous central predominance suggests a circulatory disturbance, which probably results from the simultaneous extensive reaction of the mesenchyma and the eosinophilic debris in the sinusoids.

The characteristic alteration of fulminant hepatitis is the simultaneous involvement of almost all cells in almost every lobule of the liver. In the acute form of less than 10 days' duration from the onset of jaundice, the massive necrosis seems to involve one part of the liver after the other. In areas which do not show massive necrosis, the spotty necrotic form of viral hepatitis is seen. In the parts first involved, the massive necrosis is followed by massive collapse, grossly reflected in a spleenlike appearance. When additional parts undergo massive necrosis, death results from fatal hepatic insufficiency. The consecutive bouts of massive necrosis account for the gross polymorphous appearance of the liver.

Not every patient dies in the acute stage of massive necrotic viral hepatitis. Some survive the acute stage, despite severe injury to the liver, only to succumb in the subacute or chronic stages. Others have only small patches of massive necrosis in a liver otherwise showing spotty necrosis. They may recover completely, with only small scars consisting of a few ghost lobules or collapsed areas. Only a few biopsy specimens have been obtained in the acute stage of massive necrosis [786] (Fig. 143E).

Changes in Other Organs. In necropsies of patients with massive necrotic viral hepatitis, changes in organs other than the liver are found [2083, 2085, 3651]. The extrahepatic bile ducts and the gallbladder are not involved, although the gallbladder bed is often widened by edema. The portal lymph nodes are enlarged and succulent on the cut surface and show reticulum cell hyperplasia with pigment deposition, proceeding to lymphadenosis. Severe lymphoid hyperplasia is seen in the peripheral lymph nodes also, especially in acute stages. The spleen is enlarged in the majority of cases and in about one-third of cases

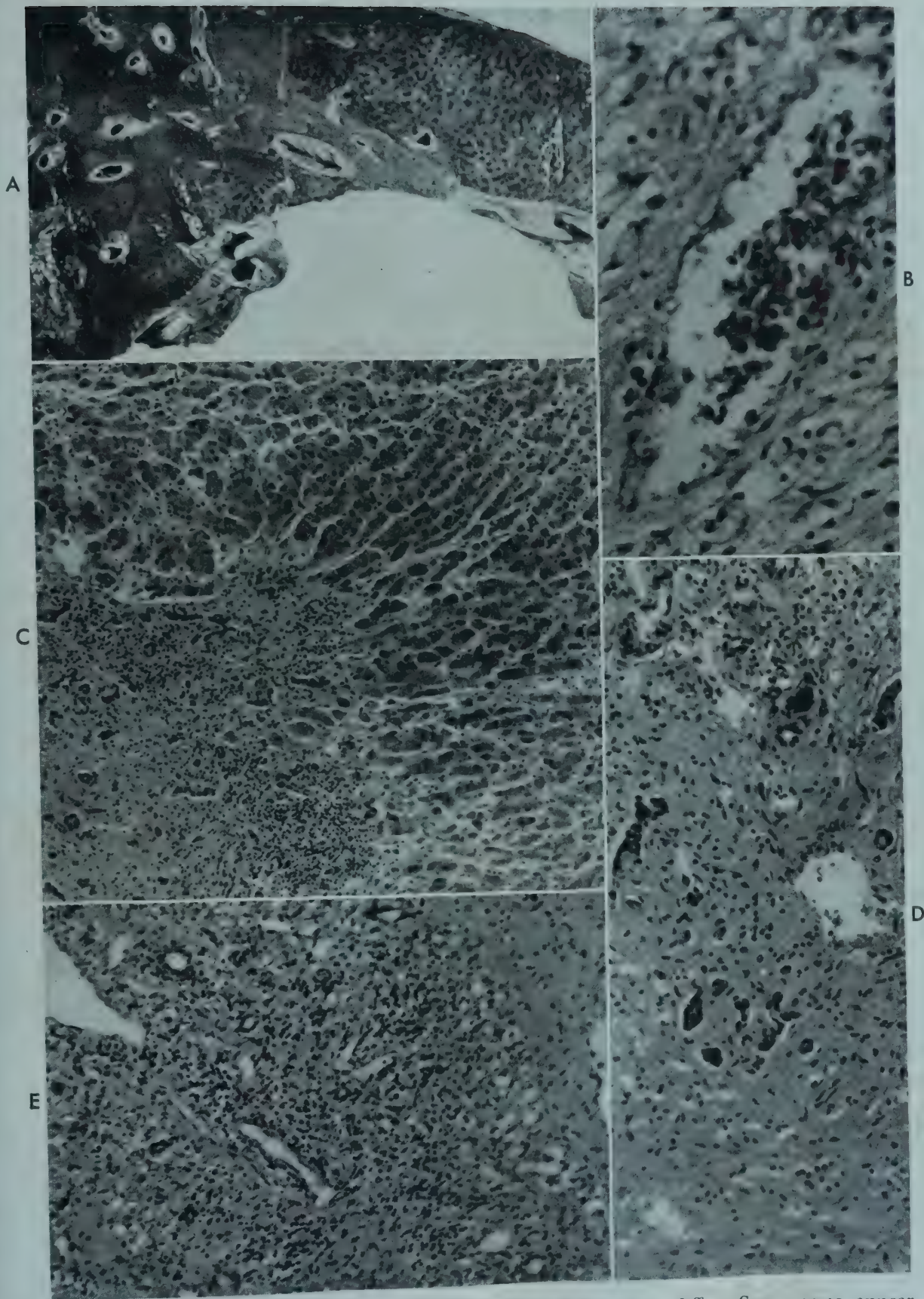


FIG. 143 A. Acute viral hepatitis; various portions of the liver differ. Some areas appear spleenlike, with no lobular markings, while in other areas, especially in the left lobe, the lobular markings are exaggerated. (Popper, H., and Franklin, M.: *Arch.Path.* 46:338, Oct. 1948.) B. Endophlebitis in central vein in acute viral hepatitis ($\times 220$). C. Border between collapsed zone and preserved parenchyma undergoing necrosis in acute viral hepatitis ($\times 75$). D. Collapse of framework in acute viral hepatitis with disappearance of hepatic cells, apparently increased numbers of ductules, and approximation of central and portal canals. H&E ($\times 105$). E. Biopsy specimen of acute massive necrosis caused by viral hepatitis ($\times 110$).

it weighs over 300 gm [2083, 2085] and shows acute or subacute splenitis with reticulum endothelial hyperplasia, deposition of iron-free and iron-containing pigment, and, rarely, small areas of focal necrosis with hemorrhage. Phlegmonous infiltration of parts of the gastrointestinal tract, especially the ascending colon, have been noted in late stages, in addition to circumscribed edema and ulceration of the esophagus [2083]. The kidneys are slightly enlarged and reveal fine fat droplets on the bases of the epithelial cells of the proximal convoluted tubules, possibly representing fat from tissue-breakdown products [2085]. Bile imbibition (cholemic nephrosis) is in the foreground in late stages. In the testes, spermatogenesis is arrested [3651]. Brain edema is common, and nonspecific degenerative changes are seen in ganglion cells. Meningoencephalitis has been reported in a few cases [2083, 3306, 3651].

Acute Cholangiolitic Hepatitis

Only a few liver biopsy specimens have been obtained from patients in whom viral hepatitis is suspected on epidemiologic grounds but in whom functional evidence of cholestasis is present without hepatic-cell degeneration. Severe bile stasis with little necrosis or conspicuous mesenchymal reaction is seen. Occasionally, acidophilic bodies are found supporting a viral etiology. Morphologic changes in the ductules are not conspicuous in early stages, and the functional findings mainly suggest the name "cholangiolitis" (see Cholangiolitis and Pericholangiolitis, Chap. 46). In later stages cellular exudate is seen around perilobular and intralobular ductules. The lesion has to be differentiated from spotty necrotic hepatitis with a definite structural and functional cholestatic component. Such a combined type of viral hepatitis is fairly frequent.

HEPATIC INJURY FROM INFECTIOUS AGENTS: CHRONIC VIRAL HEPATITIS

The natural history of viral hepatitis in its later stages is still not established. Therefore, the management of the disease in the convalescent stage is ill-defined in view of the uncertain prognosis. Moreover, discrepancies between subjective symptoms or other clinical manifestations, laboratory findings, and histologic alterations are common.

Factors Responsible for Protracted Hepatitis.

SEVERE ACUTE STAGE. The reason for lingering hepatitis is not necessarily a more severe acute stage [156, 215, 1761], and in patients with mild or even anicteric hepatitis, cirrhosis may develop [121, 135]. The situation has been compared to glomerulonephritis [1872] and rheumatic fever [231]. This does not exclude the possibility that the incidence of residual findings depends upon the severity of the acute attack [1792]. Other factors suggested are alcoholism [156], intercurrent infections including syphilis [121, 156], exercise [156, 3280], malnutrition [156, 334], such constitutional factors as age and race, endocrinologic factors as in menopausal women (see *Factors Modifying the Clinical Course of Hepatitis*, Chap. 43), and hepatotoxic factors such as anesthetics [156]. Harmful effects of alcohol [1128] and exercise [2425] in viral hepatitis have been denied by some.

PERSISTENCE OF THE VIRUS. The life span of the virus in the liver is not known. Whether protracted morphologic changes and clinical symptoms are the result of a persistent viral infection is not known, although attempts at transmission to volunteers during convalescence were unsuccessful [1420, 2419]. The persistence of the characteristic histologic lesions, whether spotty or massive necrosis and including acidophilic bodies, suggests the persistence of the virus. Some of the clinical manifestations are possibly the result of

involvement or sensitization of other organs, such as the gallbladder or duodenum [1668, 2544], or are purely psychoneurotic in nature [217].

PERSISTENT ANATOMIC CHANGES. Conspicuous anatomic changes are sometimes found in the absence of significant clinical or laboratory findings for the following reasons:

1. The recovery period of the spotty necrotic form of viral hepatitis varies; in some instances anatomic healing is complete within 1 week after subsidence of jaundice, while in others it requires months or even years. If single-cell necrosis occurs without significant degeneration of the surrounding lobular parenchyma and regeneration repairs the injury, no demonstrable functional impairment or clinical manifestation is associated with a hepatic lesion which appears active [2897].

2. Mesenchymal lesions with a cellular or connective tissue response and permanent scarring, particularly in the portal tracts, are often impressive while laboratory findings or clinical manifestations are insignificant.

3. The permanency and significance of scarring are questionable, since regression of such scars has been suggested on the basis of serial biopsies [125, 215, 786, 1872].

4. Focal areas of massive necrosis occasionally complicate an otherwise spotty necrotic hepatitis, producing focal collapse of liver tissue without clinical significance.

PERSISTENT SYMPTOMS. Persistent neurasthenic and gastrointestinal manifestations are encountered in the absence of significant structural or functional hepatic changes because the liver is only slightly involved, if at all. The hepatic tests are not sensitive enough or histologic changes are not distinctive enough to reveal the slight hepatic involvement.

INACCURACY OF BIOPSY FINDINGS. The disease process is unevenly distributed throughout the liver during the convalescent period; therefore the small biopsy specimen may not be representative of the entire organ.

SEQUELAE. The incidence of the various sequelae is not established, because most observations are based on selected material, chiefly from patients with persistent symptoms. Mass investigation of veterans showed a surprisingly low incidence of laboratory changes [2409]. In volunteers, in whom close supervision is possible, chronic forms have frequently developed [2419]. Studies on military personnel or veterans revealed that 17 per cent of patients had an abnormal convalescence [1792], and 5 per cent or less did not completely recover after 1 year [1792, 2409]. This is in contrast to studies in which cirrhosis was found in 9 of 120 cases of acute hepatitis [3040], in 12 of 49 cases [1857], and in 23 of 48 cases [215]. These differences result from the criteria used, from the collection of the material, and possibly from differences in the virus. In spotty necrotic hepatitis, complete clinical, laboratory, and morphologic recovery apparently results within 2 weeks after the disappearance of jaundice (or of the chief manifestations in the anicteric form) in about 80 per cent of cases [156, 1882].

TYPES OF PROTRACTED HEPATITIS

Several types of protracted viral hepatitis emerge from this confusing maze of information,

some of which are clearly defined; others are still controversial (Fig. 144). The following residuals, in order of decreasing incidence, have been observed: (1) neurasthenic-gastroenterologic syndromes; (2) persistent anicteric, subicteric, or icteric spotty necrotic hepatitis; (3) relapsing hepatitis; (4) persistent hyperbilirubinemia; (5) diffuse septal cirrhosis. The clinical and laboratory evidence for healing is an important clinical problem.

Complete anatomic recovery is not possible in massive necrotic hepatitis. When it remains active, it results in chronic hepatitis (subacute dystrophy or malignant hepatitis). This often occurs in epidemics [50, 231, 291, 3562] and may be caused by a specific virus strain. Massive necrosis also progresses to arrested or active postnecrotic cirrhosis. Chronic cholangiolitic hepatitis or cirrhosis of possible viral etiology is better substantiated than the acute stage (see Cholangiolitis and Pericholangiolitis, Chap. 46).

Neurasthenia and Dyspepsia

In approximately 15 per cent of patients with viral hepatitis, neurasthenic symptoms or vague gastrointestinal complaints persist for several months after the subsidence of jaundice. The neurasthenic symptoms are emotional instability, depression, weakness, lassitude, and rapid fatigue, with greatly decreased exercise tolerance and tremor [156, 217, 493, 1576]. The gastrointestinal symptoms are pain or discomfort in the right upper quadrant, anorexia, and fat intolerance

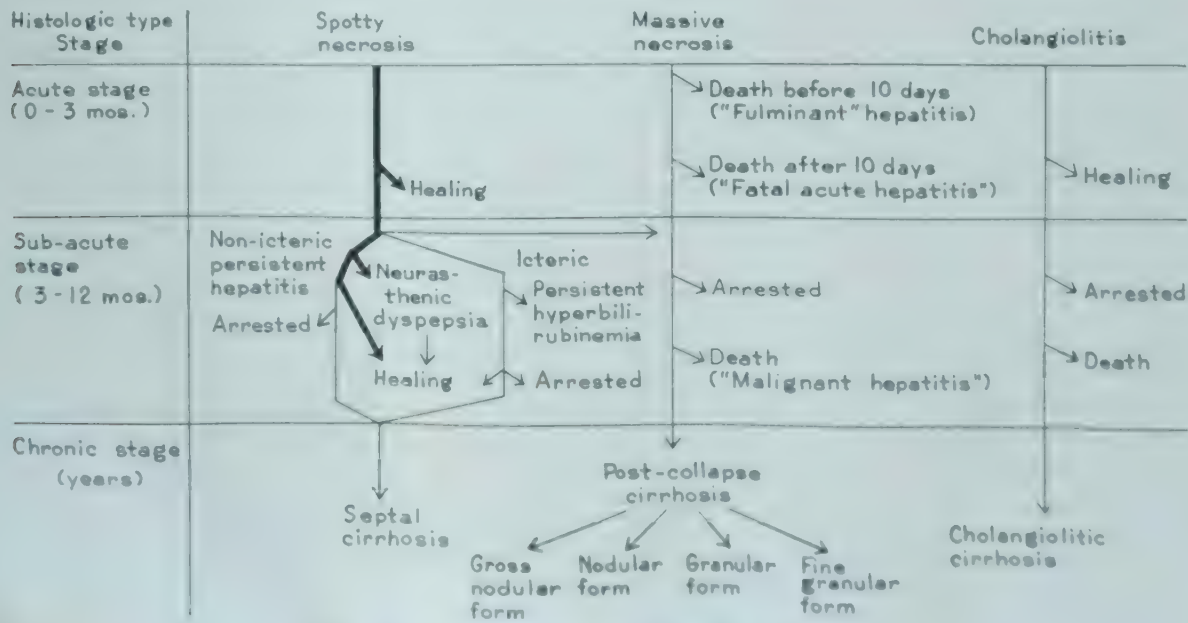


FIG. 144 Types and stages of viral hepatitis.

[856, 1792, 3373], occasionally with cramping or diarrhea [156]. The liver is often enlarged and slightly firmer than normal but not tender. Results of hepatic tests are infrequently abnormal; Bromsulphalein retention, increased urinary urobilinogen excretion, and elevated thymol turbidity are encountered [217, 1792, 1882, 2897]. In the few instances where liver biopsy was performed no abnormality or nonspecific reactive hepatitis was found, nor were some of the changes described under chronic hepatitis (see following section) [154, 217, 854, 2897, 3045].

Persistent Anicteric, Subicteric, or Icteric Spotty Necrotic Hepatitis

About 0.6 per cent of civilian patients with jaundice have clinical, laboratory, or histologic evidence indicating the presence of persistent liver disease [2409]. In military personnel this incidence is much lower, apparently because of the younger age group [3709]. Many of the chronic cases do not have a history of preceding jaundice [2409]. The clinical, laboratory, and histologic findings are not necessarily correlated.

The group is heterogeneous, the cases varying from mild, asymptomatic structural or functional abnormalities to protracted persistence of all the findings of the acute stage of spotty necrotic viral hepatitis. The majority of cases heal within 2 years. The disease entity has only recently been established [154, 156, 217, 1576, 2897, 3437]. It is also seen in children [2049, 2385].

Clinical Features. In addition to neurasthenic symptoms, pain or distress in the right upper quadrant and epigastrium is frequently noted [156, 1792, 2897, 3437]. The liver is usually enlarged and tender. Spider angiomas and palmar erythema are common, while the spleen is only rarely palpable [156, 2660, 2897]. In rare instances hypersplenism, with severe thrombocytopenia, occurs [1021].

Laboratory Findings. The most important laboratory finding is increased Bromsulphalein retention [156, 1882, 2897, 3373] associated with reduced biliary excretion of the dye [3633], although normal Bromsulphalein clearance does not exclude persistent hepatic damage. Urinary urobilinogen, zinc sulfate, and gamma globulin turbidity may be increased. The thymol turbidity is abnormal longer and more often than the cephalin flocculation [1879]. The prompt-reacting and total serum bilirubin are slightly increased, but bilirubinuria is unusual [2897]. Some patients have

deranged protein metabolism with excessive catabolism of sulfur amino acids [1767]. Anemia is not part of the picture, but atypical lymphocytes and monocytes, not the cells of infectious mononucleosis, are frequently found [156]. In some instances, evidence of hepatic-cell degeneration persists longer than the other signs; in some, mesenchymal reaction; and in others, cholestasis.

Structural Changes. The only information about the gross appearance of the liver comes from peritoneoscopic studies, in which the liver has been described as large and granular [1677]. Histologic studies in the convalescent and protracted stages of spotty necrotic hepatitis based on necropsy [2083] or biopsy material [215, 786, 854, 856, 1761, 1857, 1872, 2189, 2897, 2901, 3316] are confusing if one attempts to correlate them with clinical and laboratory findings or with the duration of the disease. If these attempts are omitted, the morphologic course of events becomes clearer, because the duration of each finding varies considerably in individual patients. Epithelial changes, such as degeneration and necrosis, intralobular mesenchymal lesions, and portal and periportal alterations, disappear at different times or persist for long periods.

PERSISTENT SPOTTY NECROSIS. Spotty necrosis may continue for several months. Usually the rate of this necrosis slows considerably, and isolated cells become necrotic while the surrounding parenchyma appears normal (Fig. 145A). The extreme polymorphism in the acute stage, especially noted in pyronin stain sections, is usually gone. Isolated acidophilic bodies and pigmented scavenger cells in an otherwise normal parenchyma are seen up to 7 months after the subsidence of jaundice and other manifestations [1872, 2901]. Regeneration keeps pace with the cell loss, and hepatic function is usually not impaired. In other instances, full-blown spotty necrosis with diffuse hepatic-cell degeneration is seen (Fig. 145B), and although regeneration replaces the loss, liver function remains impaired and jaundice often persists. The regeneration is sometimes focally accentuated more on the lobular periphery than in the central zone. This results in small nodular areas with two-cell-thick plates. Sometimes hepatic-cell degeneration and necrosis occur in waves, producing relapsing hepatitis. In some patients, especially very young ones, regeneration exceeds degeneration and the histologic findings are dominated by a spotty type of excessive regeneration with many binucleated and even multinucleated giant cells

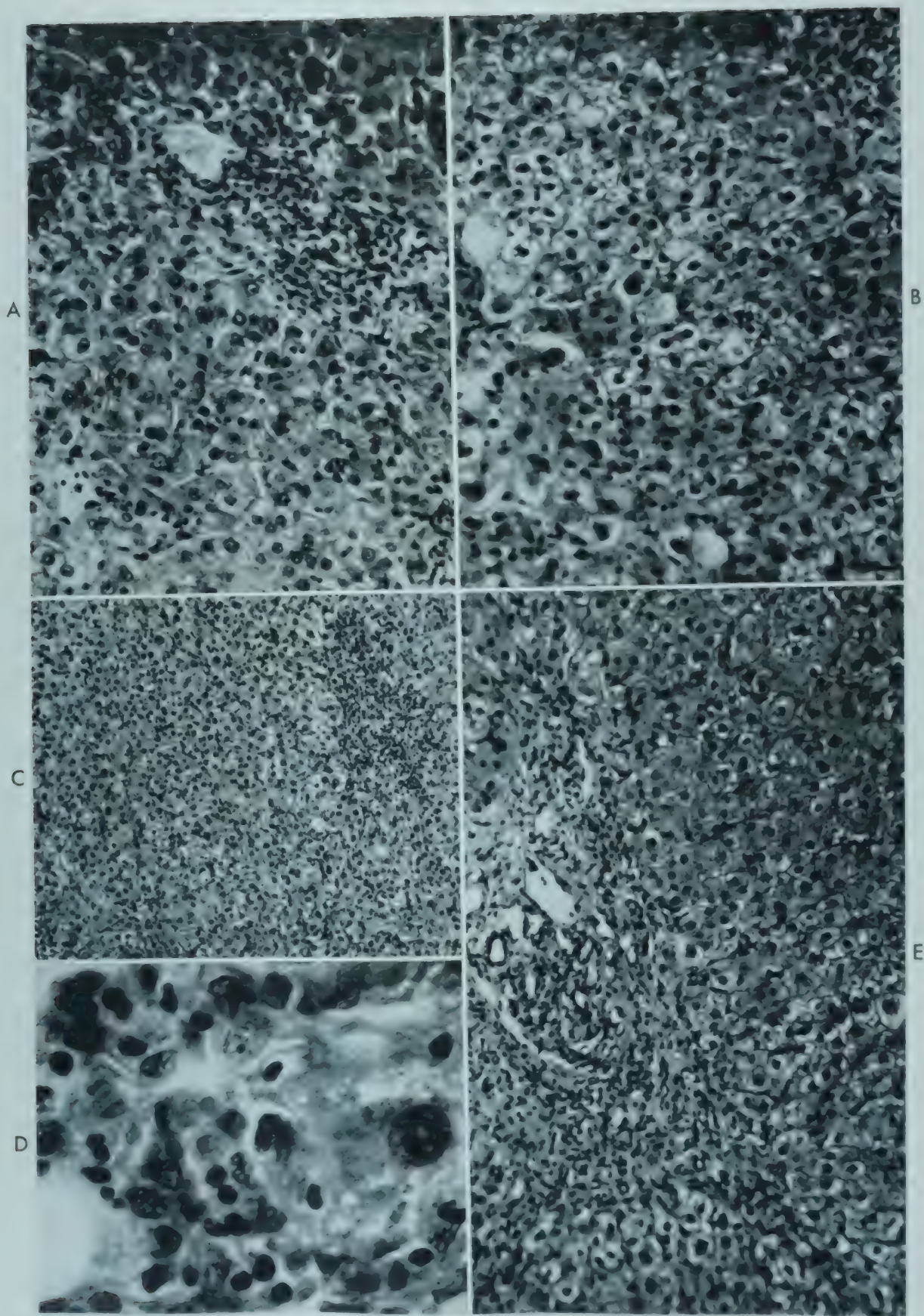


FIG. 145 Biopsy specimens of persistent viral hepatitis. H&E. *A*. Spotty necrosis and hepatic-cell degeneration, as well as portal inflammation ($\times 140$). *B*. Variation in shape and staining qualities of neighboring hepatic cells ($\times 125$). *C*. Intralobular and periportal inflammatory reaction ($\times 70$). *D*. Same as *C* at higher magnification ($\times 455$). *E*. Portal inflammation with increase in periportal and periportal ductules ($\times 125$).

[679]. Rarely, in the chronic stage, rapid destruction of the hepatic cells produces delayed massive necrosis [996, 2083] similar to that in the acute form.

The most frequent change encountered is a non-specific alteration of the hepatic parenchyma with focal necrosis—nonspecific reactive hepatitis. Fatty metamorphosis of the hepatic cells is also frequent [1576, 1872, 2189]. Bile pigment in Kupffer and hepatic cells, as well as bile plugs in the bile canaliculi, persists for an unexpectedly long time.

PERSISTENT MESENCHYMAL CHANGES. The intralobular mesenchymal changes often persist much longer than the hepatocellular changes, or are at least far more conspicuous [856, 1872, 2901]. Focal proliferation of Kupffer cells increases. They are interspersed with histiocytes and lymphocytes and sometimes also with plasma cells but only exceptionally with segmented leukocytes. This results in the formation of nodules (*Spätknötchen*) that are either homogeneous or polymorphous, with an almost granulomatous appearance (Fig. 145C, D). Pigment-containing cells are not so common as in the acute stage. Mesenchymal nodule formation is usually associated with elevated serum-gamma globulin level. The central vein is thickened [2083].

PERSISTENT PORTAL AND PERIportal CHANGES. The portal and periportal changes are usually the most conspicuous ones and seem to persist the longest. The portal tracts are infiltrated with lymphocytes, histiocytes, plasma cells, and occasionally segmented leukocytes, simulating the intralobular nodules [786, 1872, 2083, 2189, 2901] (Fig. 145E). The edema of the acute stage subsides, and the infiltration appears perilymphatic. Lipochrome pigment persists. The fixed connective tissue elements proliferate, some becoming fibroblasts [215]. The loosened connective tissue framework becomes denser, and the collagen bundles become thicker [1872]. If the limiting plate and the periphery of the lobule are destroyed, they are usually not replaced, since the existing inflammatory edema in this zone [945, 3316] compresses the framework (Fig. 146, upper left). A new limiting plate forms between the perilobular fibrosis and the parenchyma [2463], while the inflammation gradually subsides. The portal tracts appear larger, scarred, and stellate in shape because of increased portal and perilobular connective tissue [215, 786, 1761, 1872]. These changes may subsequently subside [786]. Sometimes portal cellularity persists indefinitely but

assumes a nonspecific character. The commonly encountered increased portal cellularity in the liver of otherwise normal persons examined at random is said to be residual changes of viral hepatitis [2901].

FORMATION OF COLLAGEN MEMBRANES AND REGENERATIVE NODULES. Collagen membranes often radiate from the portal tracts and sometimes from the central veins, or they may even develop independently in the parenchyma [215, 1872, 2083, 3541]. These collagen membranes enforce the framework and account for the increased firmness of the organ upon palpation. This increase in collagenous membranes [33] has been found more than 1 year after subsidence of jaundice [1872] but subsequently usually completely disappears [215]. The collagen membranes condense to form septums (Fig. 146, lower right), which sometimes seem to encircle the lobular periphery (perilobular fibrosis) (Fig. 146, upper right). In addition septums sometimes extend into the lobular parenchyma. This is usually associated with focal nodular regeneration, especially on the lobular periphery. Eventually a few small regenerative nodules are seen which are still not completely separated by connective tissue septums from the lobular parenchyma, and the lobular architecture generally remains intact (Fig. 146, lower left).

PERSISTENT DUCTULAR CHANGES. In the presence of portal and periportal inflammation and loss of the limiting membrane, the perilobular and intralobular ductules appear increased, and mitotic figures are seen in the ductular cells (Fig. 145E). In contrast to the situation in the acute stage, exudate within and around the ductules is common [1872, 3541] and desquamation of ductular epithelium is noted. These ductular changes in some instances exceed all other findings in intensity and duration [120, 996]. They disappear when the limiting plate is restored.

RESIDUAL COLLAPSE. Small areas of intralobular collapse sometimes follow focal necrosis. Residual changes after submassive necrosis entail central denuding of the framework, which then collapses. This is usually also associated with peripheral fibrosis [215, 2189, 3541] (Fig. 146, upper right). Whole lobules or even groups of lobules sometimes collapse, and in biopsy specimens "ghost" lobules, or fragments of lobules, are seen [215] (Fig. 149C). Whether these lesions ever regress is doubtful.

FUNCTIONAL CORRELATION OF PERSISTENT HISTOLOGIC CHANGES. Three main types of histologic al-

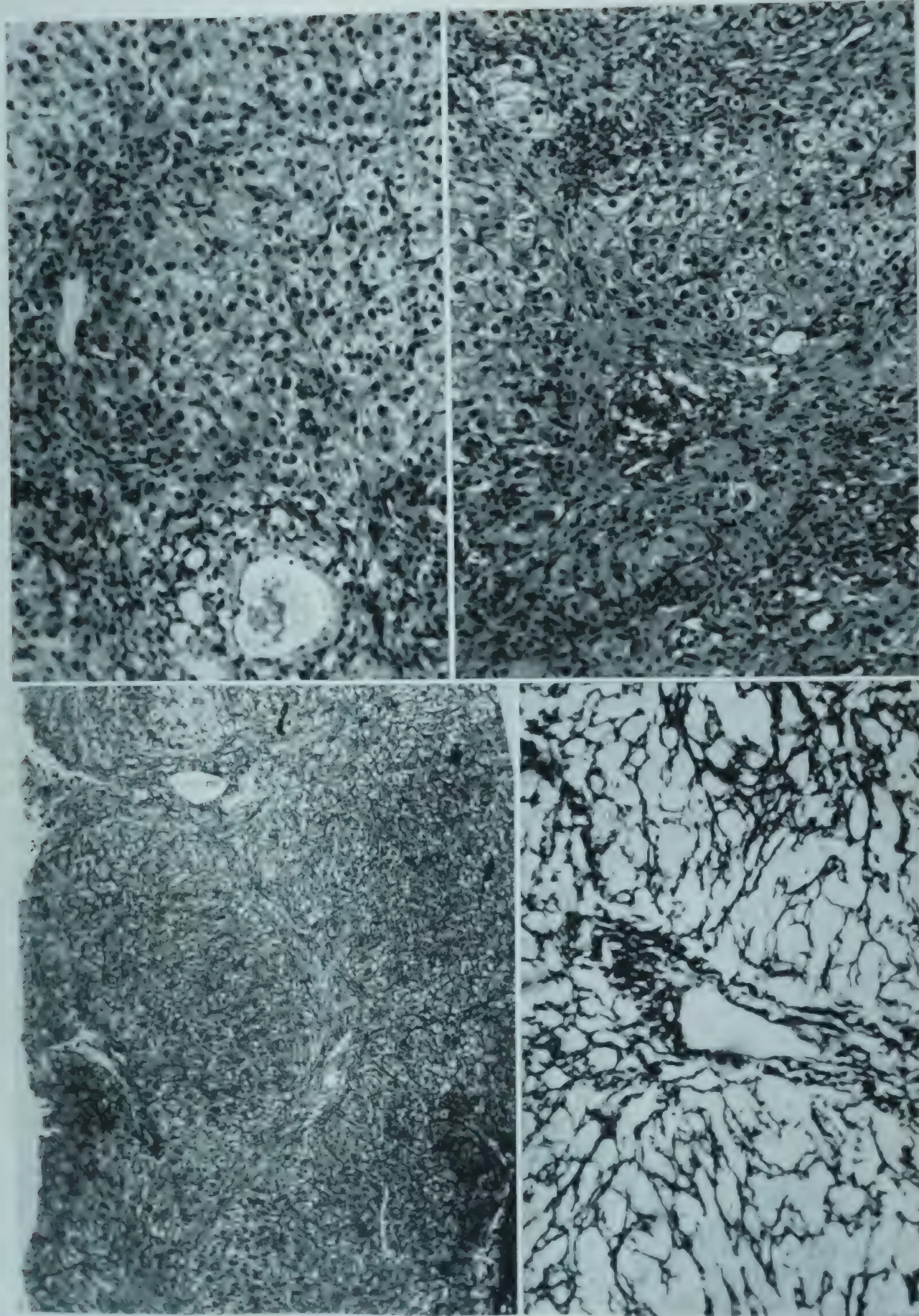


FIG. 146. Biopsy specimens of chronic viral hepatitis. *Upper left*. Periportal membrane formation with beginning periductular fibrosis. H&E. ($\times 65$). *Upper right*. Portal inflammation, periportal collapse, periductular fibrosis, and beginning nodule formation. H&E. ($\times 130$). *Lower left*. Septums connecting portal tracts with beginning dissection of the lobular patterns and focal collapse. Mallory's aniline blue. ($\times 72$). *Lower right*. Periportal membranes condensing to septums. Gomori silver impregnation. ($\times 150$).

teration persist in the protracted stage of hepatitis, each of which reveals some clinicopathologic correlations (Fig. 147). These are (1) hepatic-cell degeneration and necrosis, reflected in the alteration of the tests indicative of hepatic-cell degeneration; (2) intralobular and periportal inflammation, reflected in a persistent or increasing gamma-globulin elevation when the results of other tests tend to become normal [2636]; (3) ductular alteration, reflected in functional abnormalities characteristic of cholestasis.

INCIDENCE. The available information about the incidence as well as the time of occurrence of the various lesions is fragmentary. Formation of collagenous membranes and perilobular fibrosis have been said to occur within 2 weeks after the onset of jaundice [215, 1857, 1872, 2901]. Septums similar to those found in cirrhosis have been seen after 35 days [1857]. A grossly granular liver was noted after 90 days, but complete reconstruction has been observed only after 3½ years [215]. In sporadic follow-up studies with biopsies presumably performed on more severe cases [1857], one-third to one-half of the cases showed protracted morphologic alterations [215, 1857, 2189, 3541]. Significant permanent changes were found in less than 10 per cent of cases of chronic viral hepatitis [2359, 3541].

Recurrent Hepatitis

In some instances a second attack of hepatitis with jaundice follows the original attack within

2 weeks to 1 year after subsidence of the first attack of jaundice [1882, 2189, 2711]. The clinical picture during the interval gives no indication of the persistence of the viral infection. The histologic findings at the peak of the recurrence are identical with those of acute spotty necrotic hepatitis without any evidence of chronic changes [1872, 2189]. This suggests a new attack of hepatitis, possibly with another strain of the virus, such as B virus hepatitis following A virus hepatitis, or vice versa [2409].

Persistent Hyperbilirubinemia

In some instances, the total bilirubin level in the serum remains elevated for months or years after all other manifestations of acute viral hepatitis have disappeared [125, 156, 1576, 1882, 2660, 3373]. The elevation of the serum-bilirubin level seldom exceeds 3.0 mg per 100 ml, and the degree of visible jaundice is rarely severe. The prompt-reacting bilirubin is normal, below 0.3 mg per 100 ml, indicating that the elevation of the serum-bilirubin level is not a result of damaged hepatic function [2906]; this is also suggested by normal results of other hepatic tests and normal liver tissue in biopsy specimens [125, 1576]. Cholestasis can also be excluded as an etiologic factor. Some investigators have reported alterations in the erythrocytes, with reticulocytosis, increased fragility, and urobilinogenuria, as a cause for the jaundice [125, 1127, 1677]. Others have related it to an increased threshold for bilirubin excretion (retention jaun-

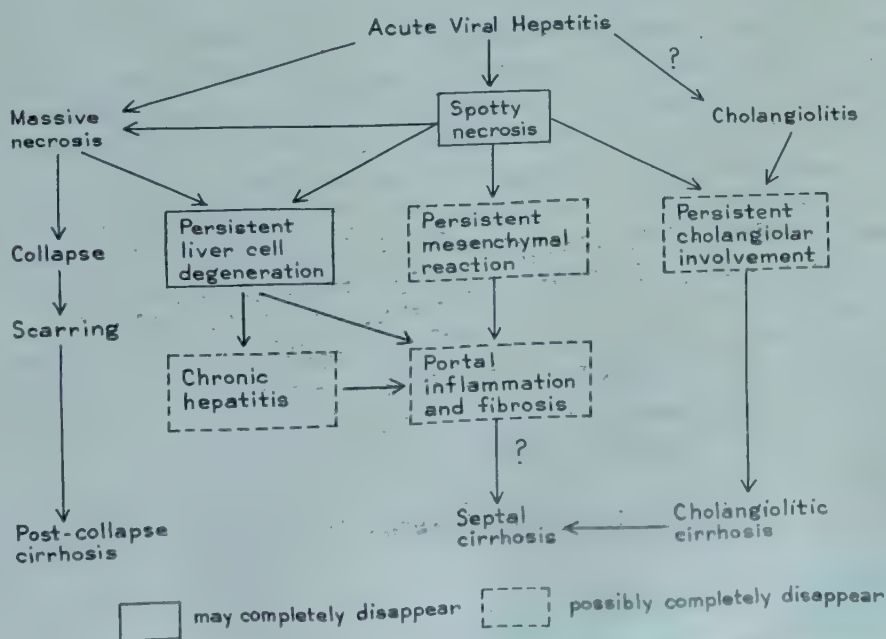


FIG. 147 Structural sequelae of acute viral hepatitis.

dice) [1576]. This finding has no clinical significance other than a differential diagnostic one.

Cirrhosis from Spotty Necrotic Hepatitis

Cirrhosis, either of the septal type or of the postnecrotic type, possibly may follow spotty necrotic viral hepatitis, although this is not established, nor is the incidence of such a transition known.

The claim has repeatedly been made that viral hepatitis never leads to a diffuse septal ("Laennec's" or "portal") cirrhosis. The term "Laennec's cirrhosis" in the German literature indicates atrophic cirrhosis of any type, including postnecrotic cirrhosis, and reports found there do not prove the point [125, 215, 231, 3316]. Some American and English observers, however, have mentioned the development of Laennec's cirrhosis, referring to a diffuse septal type after clinically established viral hepatitis [135, 716, 786, 1761, 1845, 1857, 3040, 3437]. Some emphasize that the lesion can develop without preceding jaundice from mild anicteric hepatitis [716]. In a series of cirrhotic patients from rural communities with a low incidence of alcoholism, antecedent jaundice, presumably from viral hepatitis, was fairly common [1558]. Moreover, gradual transitions from new formation of membranes and focal collapse to regenerative nodules and dissection of the lobules by septums can occasionally be demonstrated in the presence of spotty necrosis [910]. Nevertheless, few cases are substantiated by an initial liver biopsy showing acute viral hepatitis without fibrosis and by subsequent biopsies revealing stages of transition into septal cirrhosis. Even if such transition is occasionally demonstrated, other factors which damage the liver, such as dietary imbalance, can not be excluded [2083]. Since the histologic picture of viral hepatitis complicating preexisting cirrhosis is not known, the entire question of transition of viral hepatitis into diffuse portal cirrhosis remains unanswered. If diffuse septal cirrhosis is a sequela of hepatitis, it is rare. Following severe and continuously occurring focal necrosis, a postnecrotic cirrhosis may possibly develop, especially if excessive regeneration is stimulated. In rats this course of events can be produced by chronic ethionine intoxication.

Protracted Massive Necrotic Form of Hepatitis

The differentiation of chronic massive necrotic hepatitis from the acute form on one side and from

postnecrotic cirrhosis on the other is often not clear. The interval between the occurrence of massive necrosis in different parts of the liver is much longer in chronic massive necrotic hepatitis than in the acute form [2083]. The term "chronic hepatitis" indicates predominance of active necrosis, while the term "postnecrotic cirrhosis" emphasizes the end result. In many instances the lesions overlap in the same liver. Nevertheless, these stages are conventionally and helpfully differentiated. Protracted massive necrotic hepatitis has been observed in epidemics in Scandinavia [50, 231, 291] and Switzerland [3562], as well as in the American [2083] and British [3235] armed forces. Epidemics have also been observed in children [2385, 3562], with manifestations similar to those in sporadic cases [2083, 3562]. The mortality rate is high, but since the diagnosis is usually based on autopsy findings, the incidence of survival is not established. The mortality is particularly high in women who have passed the menopause; the name "malignant hepatitis" has been coined for this type of hepatitis [50, 291, 1594, 1642, 2875].

Clinical Features. Except for the terminal period the clinical picture does not necessarily differ from that of severe protracted spotty necrotic viral hepatitis. In the terminal period hemorrhagic tendencies and central nervous system manifestations are prominent. The duration varies usually from 1 to 9 months, and the preicteric period is long [1642]. In about 10 per cent of cases, jaundice never appears [291, 3562]. Pain in the gallbladder area is common. Ascites is found in about two-thirds of the cases, while edema and hydrothorax are found in about half [291, 2083, 3359].

Laboratory Findings. The laboratory findings are those of severe hepatic insufficiency with more or less severe cholestasis. The serum level of gamma globulin is greatly elevated, and values up to 8 gm per 100 ml have been reported [3718]. This accounts for the hyperglobulinemia and hyperproteinemia and abnormal results of the Takata-Ara test considered particularly helpful by the Scandinavians [50, 291, 2875].

Macroscopic Appearance. No differences can be found between epidemic or sporadic cases [231, 291, 2083, 3562]. The gross appearance of the lesion shows the transition between acute massive necrosis and postnecrotic cirrhosis [2083]. The duration of the disease can not be estimated from the gross appearance. The outer surface and cut surface vary greatly in appearance and color. The

outer surface is wrinkled and depressed in some areas, while in others it is prominent, with a tense capsule. In still other areas nodules varying in size are present. In most instances, the liver is smaller than normal, but in cases of longer duration, it is of normal size. The consistency is usually reduced almost as much as in the fulminant form. On the cut surface, three types of areas are seen, each contributing in a different degree to the gross picture of the liver:

1. In depressed, spleenlike, brown areas, the architecture is obscured. The left lobe is often mainly involved.

2. In prominent multicolored areas the architecture is preserved and the lobules appear large (Fig. 148A); their wide central zones are reddish, while the periphery varies in the same liver between yellow, green, and red, depending on blood content and degree of bile stasis. These prominent areas in some instances form the greater part of the liver, and in others, only nodules.

3. Fine or coarse nodular areas with distorted architecture are brown to yellow in color.

Histologic Changes. In the depressed areas the hepatic cells have disappeared and the framework has collapsed. In the prominent areas the hepatic cells are present or appear to be disintegrating. In nodular zones, regeneration predominates and cirrhosis is developing.

NECROSIS OF HEPATIC CELLS. In some patchy areas, the lobules are intact, although the hepatic cells show the same changes as in spotty necrotic hepatitis. In other patchy areas all or almost all cells of many lobules are necrotic, as in fulminant hepatitis. Infiltration of the branches of the hepatic vein by inflammatory cells is conspicuous, the intima may be lifted by inflammatory exudate, and small thrombi are seen [2083].

COLLAPSE AND GHOST LOBULES. In some areas all hepatic cells of several lobules have disappeared and the framework has collapsed (see Massive Necrosis, under Necrosis, Chap. 22) (Fig. 106A). The lobular arrangement is preserved, as can be seen from the normal relations of central fields and portal tracts, except that they are now closely approximated (Fig. 106B). In the denuded framework of these "ghost lobules," proliferated Kupffer cells and scavenger cells usually laden with bile and lipochrome pigments are inside and outside the sinusoids. The reticulum fibers are thickened, but otherwise the collapsed framework [2083] is unaltered, and collagenous membranes are seen

only exceptionally. The lobular framework is clearly demarcated from the thick collagenous fibers of the portal tracts (Fig. 107B).

DUCTULAR AND INFLAMMATORY CHANGES. Increased numbers of ductules are found, mainly on the lobular periphery in a fence-like fashion, but some are also seen within the lobule, even near the center (Fig. 106B). They frequently contain bile plugs. The number of plugs is especially large in cases with predominant cholestatic features. Intralobular inflammation is usually not conspicuous. The portal tracts are infiltrated with inflammatory cells, but the interlobular bile ducts and the branches of portal veins and hepatic artery are not involved.

REGENERATION. Submassive necrosis, in contrast to massive necrosis, is associated with extensive hepatocellular regeneration, which is seen in two forms.

1. After necrosis and collapse of the central and mid-zonal portions, the peripheral zone, which is intact except for destruction of the limiting plate by periportal inflammation, shows rapid regeneration, usually starting after 10 days. This is most conspicuous on the boundaries of necrotic areas. This regenerative budding is characterized by hyperchromatic nuclei and multinucleated cells, and newly formed hepatic-cell plates extend into the collapsed areas [2797] (Fig. 109B). In the intact zone, the hepatic-cell plates are often two cells thick.

2. In places, submassive necrosis reaches the periphery of the lobule. Parts of lobules are isolated, and necrotic zones extend into neighboring lobules. After collapse of the necrotic areas, the persisting fragments of one or several lobules become nodules (see Formation of Regenerative Nodules, or Pseudolobules, under Processes Common to All Types of Cirrhosis, Chap. 28) (Fig. 148E). After about 10 days [2083], hepatocellular regeneration starts, reflected by hyperchromatic nuclei, mitoses, multinucleated cells, and two- or even three-cell-thick layers of hepatic cells, as well as by an increase in the numbers of ductules. The ductules appear sometimes as small cords without lumens and sometimes as acinar structures containing bile plugs. They are surrounded by inflammatory cells. Hepatic cells and bile ducts frequently connect on the edges of the irregular patches of liver tissue. The isolated fragments of hepatic tissue become globular or undulating masses varying in size, some containing normal

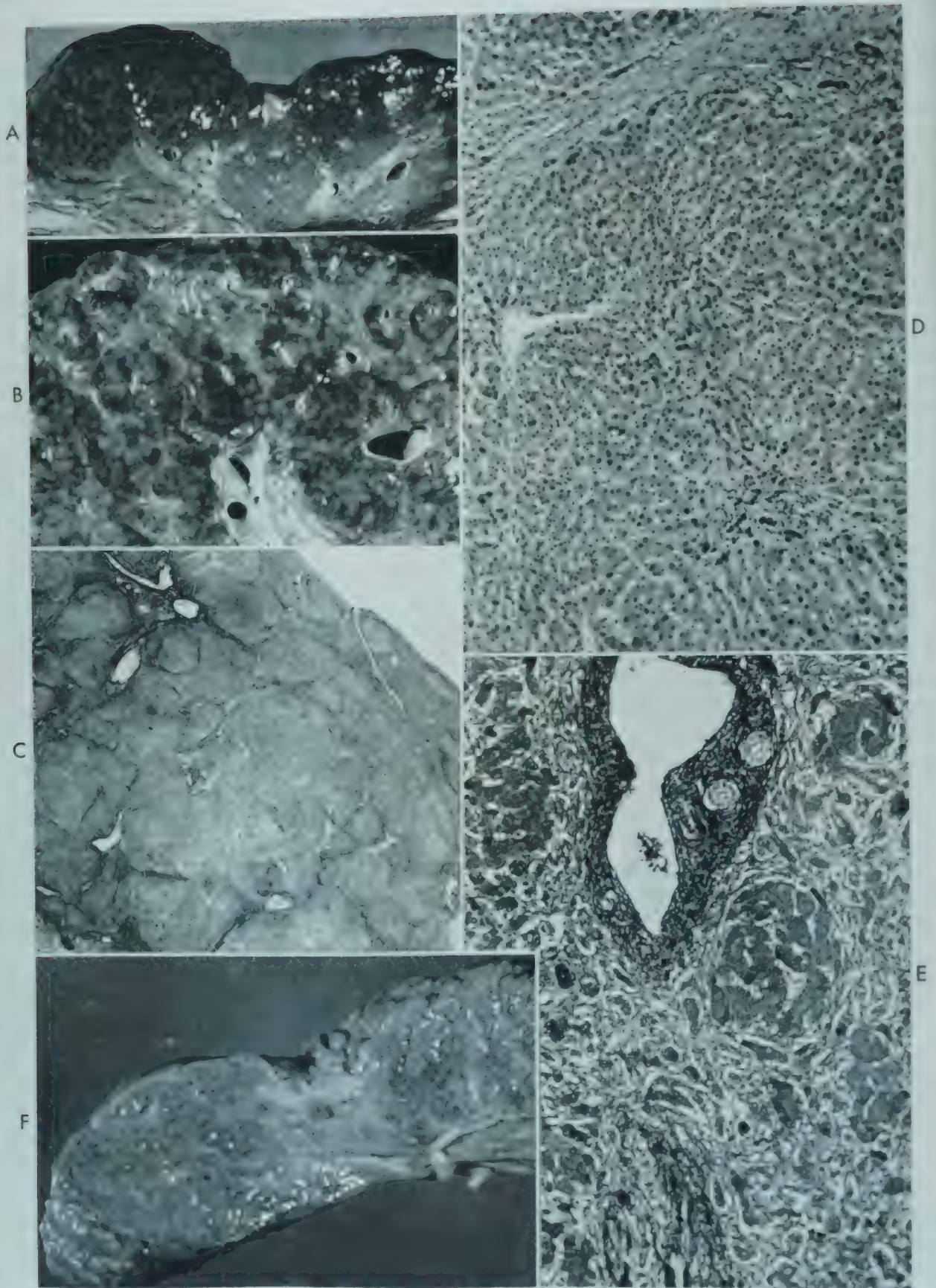


FIG. 148 A. Late stage of massive necrosis in hepatitis, with extensive collapse (spleenlike appearance) in lower portion and nodular appearance in upper portion. B. Postnecrotic cirrhosis. Note nodules of various size separated by connective tissue bands of different widths. C. Same as B in histologic section. Mallory's aniline blue ($\times 3$). D. Preserved lobular architecture in nodule of postnecrotic cirrhosis. The lobular pattern is distorted by irregular regeneration, perlobular fibrosis, and fissure. H&E ($\times 42$). E. Submassive collapse with irregular regeneration of persisting cells forming nodules in postnecrotic cirrhosis. Mallory's aniline blue ($\times 70$). F. Postnecrotic cirrhosis with large scars.

portal fields, and some larger ones containing normal lobules (Fig. 148D). A grossly nodular appearance is produced.

STRESS FISSURES. On the border of the collapsed one the intact patches of liver tissue show planes of stress resulting from the neighboring collapse (see Collapse: Postnecrotic or Postcollapse Cirrhosis, under Morphogenetic Pathways, Chap. 28) (Fig. 107C). Straight fissures extend from the area of collapse into the preserved tissue with no relation to the lobular architecture. Along these fissures, hepatic cells disappear, the framework collapses, and collagenous membranes are laid down. Eventually septums form which subdivide the lobular parenchyma and contain anastomoses or shunts between portal and hepatic vein branches [2631] (Fig. 148C). The nodular, and sometimes also the lobular, parenchyma is ischemic because of the portohepatic venous shunts in the collapsed areas or in the septums. Frequently in the center of the lobules and nodules, coagulation necrosis and disappearance of hepatic cells occur without much inflammatory reaction, apparently because of anoxia resulting from the shunts.

Morphogenesis of Subacute Massive Necrosis. Subacute massive necrotic hepatitis can be explained as necrosis involving different parts of the liver at different times and gradually encompassing the entire organ in fatal cases. This stepwise progression results in the presence of intact patches in areas of necrosis, followed by collapse, regeneration, and beginning cirrhosis formation. The extent of each of these features varies from patient to patient, as does the size of the areas of necrosis. Moreover cholestatic features occur to a variable degree. All this accounts for the great variations in the appearance of the liver. The left lobe is more extensively involved, possibly because the streamlining of blood in the main stem of the portal vein provides the right lobe with more protective nutrients absorbed from the intestine [1497] (see Streamlines of Flow, under Portal Vein, Chap. 18). The rate at which the disease progresses varies. Death results if the necrosis caused by viral infection finally destroys the greater part of the liver, or if necrosis of the regenerating or intact liver results from circulatory impairment produced by the portohepatic venous anastomoses.

Changes in Other Organs. Splenomegaly is found in about 60 per cent of the cases, but enlargement of the spleen above 400 gm is unusual. Esophageal varices are present in half the cases,

and peptic esophagitis is common, but bleeding from erosion of the varices is rare.

Hemorrhagic tendencies occur in at least two-thirds of the cases, and fatal hematemesis and melena have been reported following bleeding from erosive gastritis in 10 per cent of cases.

Plasma cells are increased in number in the bone marrow, the increase supposedly being parallel with elevation of the serum-globulin level [291]. Some marrow hyperplasia is noted, mainly in the erythroid series. Brain changes, chiefly degenerative in nature, are similar to those seen in acute stages.

Postnecrotic Cirrhosis

Cirrhosis following massive necrosis and massive and submassive collapse has been described by many authors [640, 854, 996, 1497, 2719, 3676]. It has been observed following epidemics of viral hepatitis [231, 3235, 3562] and in sporadic cases [135, 215, 854, 1880, 2463, 3037, 3040, 3316, 3676]. In comparison to other types of cirrhosis, the number of cases is small, and few larger series have been reported. The terms "toxic" cirrhosis, "postnecrotic" cirrhosis [1696, 2719], "posthepatic" cirrhosis [3040], "postcollapse" cirrhosis [2630], "coarse nodular" cirrhosis, and "multiple nodular hyperplasia" have been applied. This condition has long been considered the end stage of acute liver atrophy caused by toxic agents [2187]. Most authors attempt to differentiate postnecrotic, or postcollapse, cirrhosis from diffuse septal cirrhosis not only pathologically [135, 1201] but also clinically [135, 1497, 1880].

The massive and submassive necrosis which supposedly precedes the collapse has frequently been assumed to be viral in etiology, at least in this country. In Africa and in other areas with widespread malnutrition the same picture is observed, presumably as a result of nutritional deficiency. In some cases, the transition from acute massive necrosis, apparently on a viral basis, to postnecrotic cirrhosis has been followed. However, jaundice is not necessarily elicited in the history of the patients. In addition to antecedent hepatitis, exposure to hepatotoxic drugs and malnutrition, including alcoholism, have been reported in the history. In a significant number of cases, no etiologic factor at all is demonstrable [2719]. In such instances a preceding viral hepatitis, possibly anicteric, can not be excluded. Since the etiology of postnecrotic cirrhosis is still dubious, even if it follows hepatitis, the viral etiology of which usu-

ally can not be proved, the clinical pathologic entity is presented here with some reservations. The lesion may be a cause of symptoms and even of death. Sometimes it is merely a monument to preceding hepatic necrosis and collapse, with no clinical significance, and is then an incidental finding at biopsy or necropsy, in which signs of progression (see Rate of Progression of the Cirrhotic Process, Chap. 52) are absent.

Clinical Features. In contrast to other forms of cirrhosis, this disease occurs more frequently in females and in persons under thirty years of age. In some patients hepatic insufficiency with jaundice dominates the picture. Jaundice may begin dramatically with what appears to be acute hepatitis, or it may develop insidiously and persist throughout the course of the illness, often varying in intensity.

In other instances a latent period of many years intervenes between apparent acute hepatitis and the terminal episode. In patients in whom only liver biopsy provides evidence for postnecrotic cirrhosis, the clinical and laboratory signs of hepatic insufficiency are often meager. When significant symptoms appear, the condition of the patient rapidly deteriorates [2719]. Hepatic insufficiency usually precedes signs of portal hypertension. Ascites, esophageal varices, and hematemesis are not so common as in diffuse septal cirrhosis [135]. Spider nevi are frequently seen. Splenomegaly is present and may be associated with evidence of hypersplenism, such as neutropenia or thrombocytopenia [1880]. The liver is not necessarily enlarged, but the edge feels nodular. Fever is often observed, and abdominal pain is a frequent complaint.

Laboratory Findings. The reduction in serum albumin and the abnormal cephalin flocculation are similar to those found in any type of cirrhosis, but the total globulin and gamma globulin are very high in many instances [135, 1880, 2719]. Similarly, the thymol turbidity is more elevated than in diffuse septal cirrhosis following fatty liver. Total serum cholesterol and esters are usually reduced. The level of serum mucoproteins is characteristically very low. Serum-alkaline phosphatase activity is moderately elevated except for occasional instances with severe cholestasis. In some cases, abnormalities in the hepatic tests are entirely absent except for Bromsulphalein retention.

Macroscopic Appearance. Size, nodularity, and color of the liver vary greatly, depending upon the

degree and extent of post- and present hepatocellular injury, cholestasis, and the intensity of regeneration. The color is influenced by the degree of jaundice present. The characteristic gross features are wide bands of firm, white connective tissue irregularly traversing the liver. In these bands, vessels but no nodules can be seen (Fig. 148B). Another feature, not always well developed, is variation in the size of the nodules; in the larger ones the lobular architecture is discernible (Fig. 148C). Four main gross types can be recognized, with many mixed forms [135, 231]:

1. Gross nodular cirrhosis, or multiple nodular hyperplasia, somewhat resembling *hepar lobatum* [231] and mainly of morphologic interest. Most of the liver tissue is intact, and isolated scars or large nodules up to 6 cm in diameter are seen (Fig. 109C). Clinically only the nodular character on palpation is of interest.

2. Nodular cirrhosis, in which large nodules up to 4 cm in diameter and wide connective tissue bands dominate the picture. The surface is irregular, and from the base of deep depressions wide bands extend into the parenchyma (Fig. 148F).

3. Granular cirrhosis, in which the nodules are relatively small, up to 0.5 cm in diameter, but vary in size and are separated from each other by scars of varying widths.

4. Fine granular cirrhosis, in which the nodules are of equal size, the large connective tissue bands are missing, and differentiation from diffuse septal cirrhosis is difficult grossly and sometimes even microscopically [135, 231]. In general the left lobe shows a greater tendency for large areas of collapse [135, 1497, 1880]. Further variations are caused by necrosis, if any, and by the interference with blood and bile flow, characteristic of an active process.

Histologic Alterations. The typical features are (1) variations in extent of the cirrhotic process throughout the liver, reflected in normal architecture and almost normal-appearing portal tracts in some places; (2) broad septums consisting of collapsed parenchyma; (3) variations in the size of the regenerative nodules; (4) excessive regeneration.

NECROPSY SPECIMENS. In the broad bands, the original portal tracts and central fields are approximated but are still well discerned (Fig. 110B). All gradations from extremely thick septums to thin ones connecting portal tracts with each other or with the central fields are seen, so that in places in the same liver diffuse septal cirrhosis is ob-

erved in addition to the wide bands. Some portal tracts show inflammatory infiltration and a stellate shape, whereas others are normal. The hepatic veins are frequently flattened and may show thrombosing phlebitis. Some regenerative nodules are small (Fig. 110E), whereas others are composed of several lobules with almost normal-appearing and normally spaced portal and central canals (Figs. 110A, 148D). Regenerative features are suggested by several-cell-thick plates and by bizarre shapes of the hepatic cells. This tendency to increased regeneration also explains the relatively high incidence of carcinoma formation in this type of cirrhosis [2719, 3037]. Necrosis is found in the center of lobules and nodules in cases with clinical activity. Cholestasis may be severe. Fatty metamorphosis is seen if malnutrition complicates the course of the disease.

BIOPSY SPECIMENS. In view of the small size of the biopsy specimens, many of the features of postnecrotic cirrhosis can not be evaluated, and the diagnosis is usually only tentative. After viral hepatitis, distortion of the lobular architecture by septums connecting central with portal canals is sometimes noted. This is apparently the result of collapse (Fig. 149A). While this appearance is histologically not characteristic for postnecrotic cirrhosis, it becomes meaningful with the knowledge of the clinical circumstances. The regenerative nodules frequently show very active and irregular regeneration (Figs. 110C and D, 149B). A bizarre shape of the regeneration, varying in degree in different nodules, is suggestive, although not diagnostic, for postnecrotic cirrhosis in the liver biopsy specimen. The criteria of the degree of hepatic-cell damage, extent, and progression of cirrhosis, as described for septal cirrhosis mainly on a nutritional basis (see Functional Therapeutic Classification, Chap. 52), are applicable to these biopsy specimens. Sometimes small foci of collapse with formation of "ghost lobules" are seen in otherwise nearly unaltered livers (Fig. 149C). They are residual of massive necrosis and are without clinical significance.

Pathogenesis. The pathogenesis of postnecrotic cirrhosis appears better understood than that of most other types [3316] and has been substantiated by three-dimensional reconstructions [2630, 2631] (see Collapse: Postcollapse or Postnecrotic Cirrhosis, under Morphogenetic Pathways, Chap. 28). Any type of collapse contributes to this formation, such as (1) massive collapse with the formation of ghost lobules; (2) submassive col-

lapse resulting in irregularly arranged fragments of lobules enlarged by regeneration; (3) peripheral collapse characterized by enlargement of the portal fields at the expanse on the destroyed lobular periphery; (4) central collapse. Moreover experiments on rats fed ethionine for prolonged periods indicate that continuously occurring focal necrosis with subsequent focal collapse may eventually also lead to postnecrotic cirrhosis. This may explain clinical instances of this disease without a dramatic course and without jaundice. Although the various types of collapse may produce stress fissures in the surrounding tissue, extensive areas of the liver may be almost normal, having recovered from the acute injury responsible for the massive necrosis and the collapse. The extensive necrosis characteristic of the process is responsible for the morphological signs of bizarre regeneration. The extensive mesenchymal reaction is responsible for the frequently found high serum-gamma globulin levels. In some instances, a cholestatic component may become very prominent, and then the lesion resembles acute cholangiolitis (see Cholangiolitis and Pericholangiolitis, Chap. 46). Cases are reported in which cholangiolitic cirrhosis followed massive necrotic hepatitis [996, 1761, 3541].

Laboratory Findings during Convalescence from Protracted Viral Hepatitis

The unpredictable course of viral hepatitis in the later stages and the danger of transition into a disabling or even fatal condition make the management of the individual case difficult. For instance, physical activity while hepatic injury persists is said to be detrimental and to increase the tendency for chronicity [156, 1128]. The decision as to when to permit return to normal activity is made more difficult by the lack of correlation between clinical, laboratory, and histologic findings in chronic viral hepatitis. In the attempt to steer safely between the danger of chronicity and prolonged unjustified morbidity, a course is recommended which still requires further confirmation by follow-up studies. As long as tenderness of the liver or abnormal results in two of four groups of tests persist, physical activity should be restricted. These groups of tests are (1) Bromsulphalein retention above 8.0 per cent in 45 minutes; (2) increased gamma globulin and zinc sulfate turbidity, thymol turbidity, or cephalin flocculation; (3) elevated prompt-reacting bilirubin or increased urinary urobilinogen; (4) low cholesterol ester

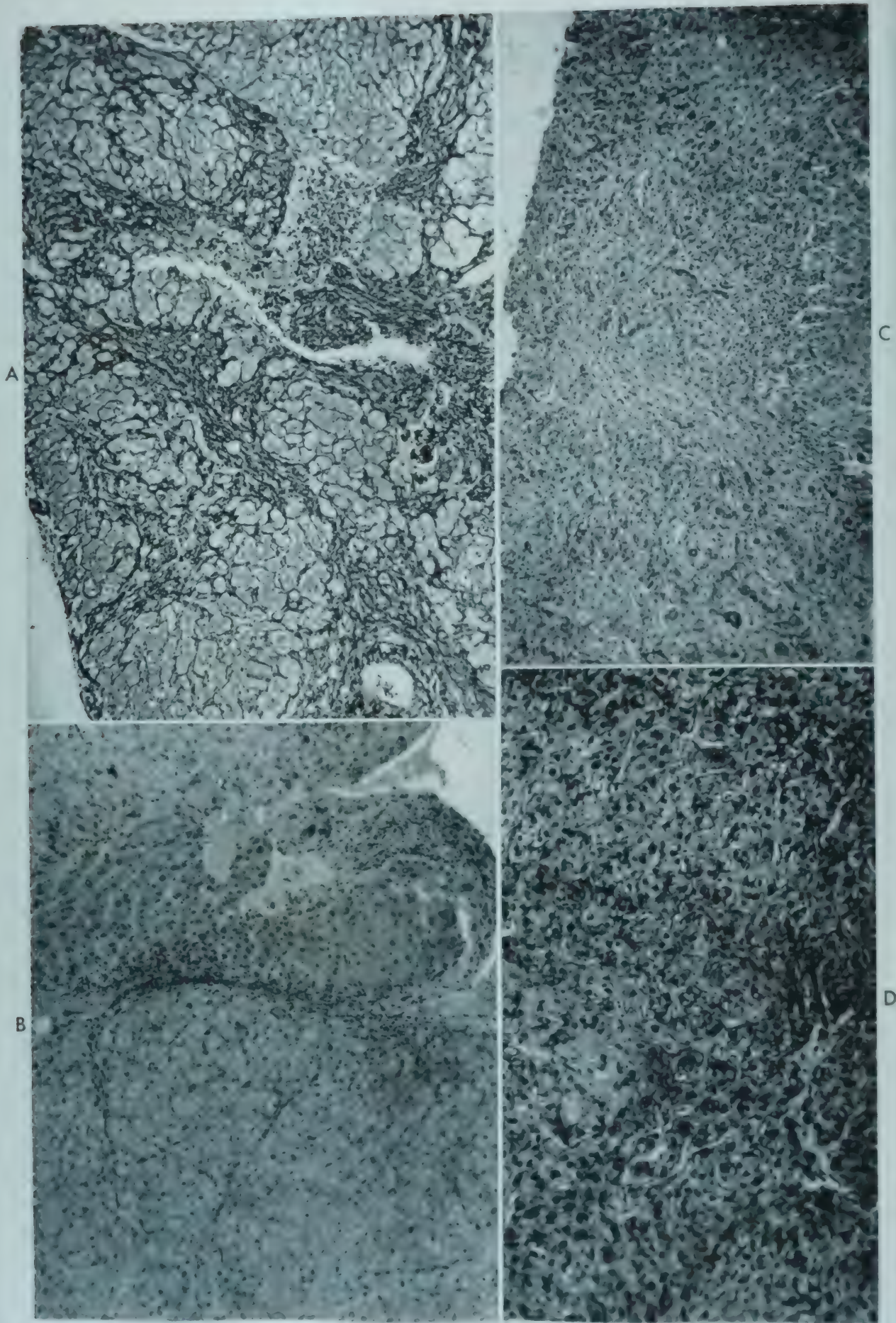


FIG. 149 Biopsy specimens. A. Postnecrotic cirrhosis following viral hepatitis. Gomori silver impregnation ($\times 110$). B. Nodules with irregular regeneration in postnecrotic cirrhosis. H&E ($\times 115$). C. Circumscribed areas of massive collapse following viral hepatitis. H&E ($\times 95$). D. Infectious mononucleosis showing diffuse hepatic-cell degeneration and inflammation similar to viral hepatitis. H&E ($\times 125$).

tio or serum cholinesterase. When the results of these tests return to normal, the patient is allowed gradually increasing activity. If the results remain normal after 2 weeks of activity, the patient may be dismissed from observation. Repeat examinations at 3 and 6 months have been recommended [409].

If abnormal findings in any one of the four groups persist two months after the onset of jaundice, liver biopsy is indicated. If nothing more than a nonspecific reactive hepatitis is found, gradual return to normal activity is recommended; but if conspicuous hepatocellular damage is present, activity should still be restricted. If the laboratory findings do not revert to normal after an additional 2 months, with or without activity, another biopsy is indicated.

If the results of only one of the groups of laboratory tests remain abnormal, activity may be permitted; but if the abnormality persists after 3 or 4 months, liver biopsy is indicated. Increase in serum gamma globulin or zinc sulfate turbidity may disappear if corticosteroids are administered. In patients with persistent neurasthenic or gastrointestinal symptoms, liver biopsy is recommended for confirmation of the diagnosis.

Viral vs. Toxic Hepatitis. Viral hepatitis involves the liver heterogeneously grossly, microscopically, and in time. This results in great variations in the clinical picture and in confusion in protracted stages. In contrast, most toxic hepatic injuries produce a more uniform change in the liver. This results in differences between acute fatal viral and toxic hepatitis, shown in Table 50.

Table 50 Characteristics That Differentiate between "Viral" and "Toxic" Hepatic Necrosis

Condition	"Viral" type	"Toxic" type
Distribution of damage	Diffuse, with central predominance	Usually zonal
Cell death	Rapid	Gradual
Large cell remnants or fragments without nuclear staining but with shadows of cellular structure (ghost cells), often with diffuse imbibition of bile	Absent, except for rapidly disintegrating cells in very early fulminant form	Present
Cytoplasmic clumps of coagulated protein	Usually absent	Often extensive
Hyalinization of cells due to diffuse coagulation necrosis (Councilman bodies)	Present	Absent
Fatty changes	Little if any (small droplets)	May be severe (small and large droplets)
Denudation of connective-tissue framework	Reactive	Silent
Rupture of framework	Absent	Occasionally present
Edema with widening of perisinusoidal space	Absent	Frank except in fatty form
Cellular infiltration	Chiefly mononuclear cells	Less intense than in viral type; polymorphonuclear cells may predominate
Bile-containing macrophages	Many	Few
Phagocytosis	Extensive	Moderate or slight
Fibrin thrombi	Rare	Rather common
Hepatic-cell regeneration in form of pseudo-bile-duct proliferation	Common	Often absent
Size of liver	Usually reduced	Slightly enlarged in central necrotic form, moderately to greatly enlarged in fatty form
Uniformity of picture	Often absent	Present
Cut surface	Spleenlike or focally exaggerated lobular pattern	Diffusely obscured or exaggerated lobular pattern
Consistency	Much reduced	Slightly reduced
Greasy appearance	Absent	Occasionally present

HEPATIC INJURY FROM INFECTIOUS AGENTS: DISEASES OTHER THAN VIRAL HEPATITIS

Hepatitis Caused by Infectious Mononucleosis. The occurrence of jaundice in infectious mononucleosis has long been known and was thought to result from compression of the common bile duct by lymph nodes. Recently necropsies, biopsies, and modern hepatic tests proved it to be caused by hepatitis, structurally and functionally. This creates the problem of differential diagnosis from viral hepatitis.

Little is known about the etiology and epidemiology of infectious mononucleosis. It is probably viral in origin but differs from infectious hepatitis in that it is not transmissible to human volunteers via blood, stools, or nasal washings [956]. Isolated instances suggest that the incubation period is 5 to 8 weeks [2542] and that the contagiousness of the disease is low [958, 1506].

Incidence of Hepatic Involvement. The incidence of jaundice varies from 0 to 13 per cent in different series, averaging from 3.6 to 6.6 per cent in larger ones [1506, 3218]. Tender or enlarged livers have been found in 34 per cent of patients [219], and some are admitted to the hospital with a diagnosis of infectious hepatitis [3218]. Subclinical hepatic impairment has been reported in many patients [219, 614, 761, 3518], and it has been claimed by some to exist in all [956], although this has been denied by others [2958].

Clinical Hepatic Manifestations. When present, jaundice lasts 7 to 25 days, usually beginning in the latter half of the second week of the illness [1506, 3218]. The longest duration of jaundice reported seems to be 11 weeks [9]. It is more often present in patients with gastrointestinal symptoms than in those with pharyngeal symptoms. Although the occurrence of a chronic stage is unsettled as far as laboratory evidence is concerned, many patients with jaundice develop a postinfection neu-

rasthenia and dyspepsia similar to those following hepatitis.

Laboratory Findings. HEMATOLOGIC DIFFERENTIAL DIAGNOSIS FROM VIRAL HEPATITIS. Atypical lymphocytes or monocytes characteristic of infectious mononucleosis do not exceed 10 per cent in viral hepatitis [2034], although lymphocytosis and monocytosis are common [156, 1505] (see Acute Icteric Period, under Laboratory Findings, Chap. 43). Heterophil agglutination below 1:896 must be confirmed by specific absorption tests [1940], because agglutinations up to 1:448 have also been found in infectious hepatitis [874, 1940], with most authors reporting either negative results [156, 3721] or titers only up to 1:56 [235, 1425]. Most of the increased titer in hepatitis is caused by the normally present Forssman antibody [1940]. The appearance of specific antibodies may be delayed for several weeks in some cases of infectious mononucleosis, causing diagnostic confusion with viral hepatitis [144].

HEPATIC TESTS. The maximal abnormalities in the results of the hepatic tests occur within 2 to 3 weeks after the onset of the disease. They are not correlated with the heterophil agglutination [1661]. They usually remain abnormal for 2 to 6 weeks but may persist for 3 months [761]. Chronic hepatitis [219, 614] and even cirrhosis [1941] have been claimed on the basis of laboratory findings, but these findings have been questioned [1699]. In some series the cephalin flocculation was more often abnormal than thymol turbidity [417, 956, 2576]; in others the condition was the reverse [1118, 1661] (Table 51). Electrophoretically, abnormal protein distribution was found in all cases [3203], but the A/G ratio was seldom abnormal [219]. The elevated serum-alkaline phosphatase level frequently reported

[956, 1118, 1661, 2576] indicates the presence of cholestasis in addition to the hepatocellular degeneration reflected by the other tests.

Table 51 Number of Cases and Percentage of Abnormal Results of Various Hepatic Tests in Infectious Mononucleosis

Test	No. cases	% abnormal results
Cephalin flocculation.....	224	81
Thymol turbidity.....	246	64
Zinc sulfate turbidity.....	15	73
Bromsulphalein retention.....	142	67
Alkaline phosphatase.....	56	65
Cholesterol esters.....	29	48
Urinary urobilinogen.....	154	33
Prothrombin time.....	21	29
Serum bilirubin.....	249	25

Sources: Evans [956], Jordan and Albright [1661], Peterson [2576], Schultz and Hall [2958].

Structural Hepatic Alterations. A limited number of necropsy reports is available on patients dying from one of the complications of infectious mononucleosis. In almost all instances, nonspecific and somewhat variable hepatic alterations were reported [705, 3560]. The most uniform finding was widespread infiltration with lymphoid cells in nodular form in the sinusoids as well as in the portal tracts and in the capsule. Diffuse crowding of sinusoids by lymphoid elements has only exceptionally been described [1699].

Biopsy findings now available in increasing numbers permit a better evaluation of the structural changes [148, 1747, 3404, 3452]. The changes in patients with jaundice or hepatic involvement are similar to those in mild viral hepatitis except that abnormally shaped lymphocytes with indented nuclei are seen in the sinusoids and participate in the inflammatory reaction in the lobular parenchyma and in the portal tracts. Spotty necrosis with acidophilic degeneration and necrosis occurs even in the absence of jaundice (Fig. 149D). Acidophilic bodies are also extruded from the continuity of the hepatic-cell plate. Central necrosis is not seen, diffuse degeneration is not conspicuous, and the hepatic-cell plates remain orderly. Regeneration with the presence of binucleated cells may be even more conspicuous than

in viral hepatitis, in view of the low-grade degenerative and necrotizing changes. The mesenchymal cell accumulation around necrotic hepatic cells also resembles virus hepatitis except for the atypical lymphocytes. Kupffer cell mobilization is conspicuous, and inflammatory cells accumulate in the portal tracts, with a tendency to show streaklike extensions into the parenchyma. This is associated with increase in ductules, which rarely become as numerous as in viral hepatitis. Bile casts and bile pigmentation occur in the center of the lobule in jaundiced patients. Even slight portal fibrosis is rare. In mild instances of the disease, especially without hepatic involvement, only nonspecific reactive hepatitis is found [1507].

The development of the lesion also simulates that of viral hepatitis; in the first 5 days after the onset of the disease, the histologic changes are minimal and are chiefly confined to the parenchyma. The peak occurs between 10 and 30 days after onset; afterwards the lesion regresses, but isolated acidophilic degeneration and evidence of regeneration can be seen for several months. Bile casts also appear late in the disease. In protracted cases, periportal infiltration and Kupffer cell activity persist for more than 60 days. The histologic signs of significant hepatitis often disappear while the clinical symptoms persist [3452]. The irregularity of the histologic appearance explains the poor correlation with the results of the hepatic tests, in which elevation of serum-gamma globulin level seems to predominate.

Yellow Fever Hepatitis

Yellow fever is a viral disease with an incubation period of 3 to 6 days, occurring mainly in West Africa and Central and South America. The urban form is transmitted from man to man by females of the mosquito *Aedes aegypti*. The jungle form has an animal reservoir, probably made up of monkeys. The disease confers permanent immunity. Yellow fever can be transmitted intracerebrally to white mice. The virus and antibodies to it can be demonstrated by neutralization tests.

Clinical Manifestations. Yellow fever appears (1) as a mild grippelike disease of short duration; (2) as a severe febrile infection with headache, backache, photophobia, facial congestion, and gastrointestinal discomfort with vomiting and albuminuria; (3) or as an overwhelming toxemia with severe hepatic and renal failure. Hemorrhagic tendencies are possible as an expression of hepatic failure. Other manifestations include severe hema-

temesis, headache, prostration, shock, and oliguria. The fever and pulse rise initially. After about a week the fever slowly subsides; it is sometimes followed by a secondary rise while the pulse rate becomes abnormally slow. In patients with toxemia, the mortality rate is very high. Death is more often caused by toxic nephrosis than by hepatic necrosis.

Laboratory Findings. The diagnosis in milder forms depends upon the serologic demonstration of specific antibodies, and not on hepatic tests. Intensive studies of hepatic function have been carried out in animals; impairment was found shortly before death, although the icterus index had risen earlier. In human yellow fever, the serum-bilirubin level rises from the third to the sixth day and remains elevated about 1 week. In nonfatal cases jaundice is mild. Results of flocculation tests are often abnormal, and prothrombin deficiency parallels the severity of the illness [927]. In fatal cases the serum-cholesterol level is low.

Structural Changes. Information about the hepatic changes is available only from autopsy specimens and from liver tissue obtained postmortally by puncture (viscerotomy), which is performed on a large scale in Brazil to identify outbreaks of the disease. The degree of liver damage in earlier stages or in the milder types is not known.

The gross picture of the liver does not suggest severe changes, and the organ is soft and yellow, especially after the blood has exuded after removal from the body. The microscopic appearance is often obscured by post-mortem changes [927, 1397, 1805]. The histologic alterations include (1) degenerative changes of the hepatic cells with loss of glycogen, coagulation of the cytoplasm, and fatty metamorphosis; (2) nuclear inclusion bodies; (3) spotty necrosis with scattered acidophilic degeneration. This progresses to acidophilic necrosis, with the formation of acidophilic bodies, which are refractile and contain a pyknotic nucleus or none at all. Many of these round bodies are found in the tissue spaces separated from the hepatic cell plates, and they are the classical Councilman bodies. They differ from similar bodies in other viral hepatic diseases in that they often contain fat droplets or brown pigment [3425]. The areas of spotty necrosis sometimes merge in the mid-zone of the lobule, leaving the immediate center and periphery of the lobule intact. Inflammation is in the background, owing possibly to the rapidity with which the disease progresses. The reticulum framework is intact, and complete recovery

occurs without fibrosis in experimental animals [1805]. Focal hemorrhages are common. The findings, especially the acidophilic bodies, have been considered quite diagnostic, although in burned patients treated with tannic acid similar findings have been reported [210]. Lesions in other organs include toxic nephrosis with fatty changes, myocardial degeneration, and hemorrhagic diathesis, any of which may be responsible for a fatal outcome.

Other Types of Hepatitis Produced by Viruses

Herpes Simplex Hepatitis. Herpetic hepatitis occurs in rare instances in newborn or young infants as a result of invasion of the blood stream by the virus [3735]. In the newborn infant this develops without obvious cutaneous lesions; in older infants it is often associated with gingival stomatitis. The increased susceptibility of infants has been compared to the ability of embryonal tissue to support viral growth in cultures. In the liver intranuclear inclusion bodies are found in the parenchymal cells, with margination of the nuclear chromatin and ballooning of the cytoplasm. In addition, grossly visible areas of coagulation necrosis, varying in size and devoid of inflammatory reaction, are seen. In the necrotic areas, the vessels are also necrotic [3735].

Rift Valley Fever Hepatitis. Rift Valley fever is a virus disease of sheep and other animals in the Rift Valley of Kenya in Africa. In man, it causes a transient febrile disease. In animals confluent spotty necrosis similar to that caused by other hepatotropic virus diseases, especially yellow fever, is seen. Homogeneous acidophilic bodies also are seen.

Cytomegalic Inclusion Hepatitis. This rare generalized disease chiefly involves the kidneys and lungs and is caused by the salivary gland virus. Nuclear and cytoplasmic inclusions are found in the hepatic cells and bile duct cells in both infants and adults. The cells enlarge, and their subsequent degeneration and necrosis lead to an inflammatory reaction which proceeds to fibrosis [3670, 3671].

Viral Hepatitis in Animals. In various animals, types of viral hepatitis occur which are not related to human viral hepatitis. The animal kingdom is not a reservoir for human viral hepatitis. In horses, hepatitis develops several months after administration of serum apparently comparable to human serum hepatitis [203]. This seems to differ from the acute hepatic atrophy in South African horses, which may be the result of ingestion of toxic mate-

rial. In dogs, structural changes resembling those in human viral hepatitis, even with numerous acidophilic bodies [2847], are produced by a virus which can be grown in tissue cultures [460]. The extensive necrosis and nuclear inclusion bodies differentiate it from human hepatitis. The spread of the virus throughout the cell from the virus-containing nuclear inclusion has been studied [601]. In mice, viruses produce hepatitis [788] and leukemia associated with hepatic changes resembling hepatitis [2423]. In racoons, a fatal hepatitis is seen which is similar to human hepatitis in its morphologic aspects and which can be transmitted to ferrets [1748].

Leptospiral Hepatitis (Weil's Disease)

Weil's disease, infectious or spirochetal jaundice, is caused by the spirochete, *Leptospira icterohaemorrhagiae*, which is world-wide in distribution. It is transmitted by contact with rats and occasionally mice [1943, 2329]. These animals are the reservoir host, and their excreta contaminate areas of stagnant water. *Leptospira* are threadlike, regular, fine coils 8 to 12 μ long and 0.2 μ wide, with hooked ends. The organisms are flexible and rotate rapidly, as seen in the dark-field microscope, where care must be taken to differentiate them from nonpathogenic pseudoleptospiras. They can be stained with Giemsa solution in thick films and impregnated with silver in tissue sections. They are cultured aerobically in Noguchi medium containing rabbit serum. Young guinea pigs and hamsters are used for animal inoculation by the intraperitoneal route. These animals become jaundiced, and their blood and tissues reveal leptospiras on dark-field examination. Persons in contact with rats or contaminated water, particularly miners, workers in sewage disposal plants, stockyard workers, ditch or tunnel diggers, or fish cutters become infected by the gastrointestinal or respiratory route as well as through skin abrasions.

Stages. The severity of the disease varies greatly. A mild, grippelike illness of short duration without jaundice is more common than the full-blown disease. The latter develops abruptly after an incubation period of 6 to 12 days with a septicemic stage lasting 3 to 10 days. During this stage the clinical manifestations are those of an infectious disease with high fever, without predominating involvement of any organ. The differential diagnosis includes typhoid fever. Headaches, abdominal pain, prostration, muscle cramps,

and conjunctivitis develop. A nonproductive cough suggests a bronchitis. Hemorrhage tendencies are reflected in dermal and conjunctival petechiae and epistaxis. Jaundice, if present, deepens rapidly. Leukocytosis is present, and the urine contains albumin, increased urobilinogen, and sometimes bilirubin. *Leptospiras* can be demonstrated in the blood.

Subsequently the fever subsides, but in severe cases a second stage sets in, characterized by severe hemorrhagic tendencies, renal injury with azotemia progressing to uremia, central nervous system manifestations with increased protein, cells, and sometimes leptospiras in the spinal fluid [585], myocardial damage with hypotension or hepatic injury with jaundice [111, 2772]. In this stage differentiation from severe viral hepatitis may become a problem, but usually the severity of the renal and muscular changes points to Weil's disease. Muscle biopsy reveals necrosis with limited perifocal inflammation [3036]. Sometimes fever recurs toward the end of this toxic stage. The demonstration of leptospiras in the blood is difficult, although it is easy in the urine.

After the third week, the third stage of slow convalescence begins. Serologic methods such as agglutination, lysing, complement-fixation, or neutralization tests become helpful. Antibodies can be demonstrated in the blood for prolonged periods. Therapy is mainly based on use of antibiotics, such as penicillin.

Functional Alterations of the Liver. Hepatic involvement is not a constant feature of Weil's disease. Involvement of other organ systems is usually more significant from diagnostic and therapeutic points of view. The mortality rate depends upon the number of mild and transient cases included; in fully developed cases it reaches 30 per cent [111]. The cause of death in the fatal cases is most commonly acute interstitial nephritis with some tubular necrosis (toxic nephrosis) and bile imbibition of the tubules associated with severe azotemia [2127]. *Leptospiras* are found in large numbers in the kidneys. Myocardial failure, pneumonia, and, rarely, meningitis cause death. Hepatic failure has not been established as a cause of death, although the severe jaundice occasionally seen would suggest it. The incidence of jaundice depends upon the inclusions of transient and milder cases which do not become jaundiced. When these are excluded, the incidence is as high as 95 per cent [2772]; others report only 40 per cent [111]. The jaundice is in part caused by

hepatic-cell damage, but hemolysis from hemorrhage into the tissues may be an important contributing factor [111], particularly since the degree of jaundice does not parallel hepatocellular damage. In patients with jaundice, icterus indexes up to 300 have been observed. Abnormal cephalin flocculation, thymol turbidity, high serum-gamma globulin level, and moderately increased alkaline phosphatase activity are found [569, 2772, 3203] mainly in the jaundiced patient and reach a peak in the second or third week. In general the clinical diagnosis is confirmed by microbiologic rather than biochemical laboratory procedures.

Structural Alterations of the Liver. Pathologic findings are mainly based on autopsy specimens [111, 1397, 2713, 2772] and on only a few biopsy specimens [2501]. Most of the alterations stressed in the literature seem to be nonspecific changes, found in any severe infectious disease, or the result of terminal shock. Central necrosis is frequently reported but is probably caused by shock and uremia. Focal necroses do not occur regularly in man [3673], but they were extensive in a fetus with an intrauterine infection [1151]. Portal inflammatory changes associated with what has been called "cholangitis" are found, as in many infectious diseases. However, the presence of leptospiras in the liver suggests that some changes are not solely the result of a nonspecific reaction. Diffuse degeneration of the hepatic cells and swelling of Kupffer cells appear to be more severe than in other infections or intoxications. It is not associated with significant fatty metamorphosis. The most conspicuous finding is fragmentation of the hepatic-cell plates [943, 1397] associated with oozing of bile from the ruptured bile canaliculi. Fragmentation is usually an agonal or postmortal process, but the severe degree of dissociation of the hepatic cells seen in this disease suggests that either some dissociation precedes death or an intravital loosening of the intercellular connections of the hepatic cells makes them more susceptible to postmortal disruption. The diffuse nonspecific hepatocellular degeneration is the morphologic finding best correlated with the functional evidence of hepatic injury. Residual hepatic fibrosis or cirrhosis has not been reported.

The morphologic findings support the idea that the liver is not the main site of the disease. The therapeutic use of methionine in animals does not influence the survival rate [3673].

Canicola Fever. Canicola fever due to *Leptospira canicola* is a common disease in dogs, pro-

ducing nephritis, occasionally associated with jaundice. A few cases have been reported in man, most of which resulted from contact with infected dogs. Jaundice is less common than in Weil's disease [2772, 2813]. In one child, severe hepatic necrosis with inflammatory reaction has been reported [1958]. Other leptospira causing infections in animals are pathogenic in man in various parts of the world other than the North American continent [2865].

Relapsing Fever

In relapsing fever jaundice is sometimes encountered. Areas of focal necrosis are found mainly in the central or mid-zonal portion of the lobule. With silver impregnation, spirochetes can be demonstrated in the sinusoids and in the perisinusoidal tissue [71].

Malarial Hepatitis

The malarial parasites supposedly lodge in the liver rather than in the spleen between attacks in chronic falciparum infections. Erythrocytic forms of *Plasmodium vivax* in the liver have been described [3058].

The effect of malaria upon the liver is seldom so severe that the liver becomes the main organ of clinical concern, except in late stages when cirrhosis supposedly develops from repeated attacks.

Laboratory Findings Pertaining to the Liver. The hepatic involvement in malaria depends upon the severity of the infection and upon the plasmodium responsible. In therapeutically induced malaria, the serum-bilirubin level rises slightly without bilirubinuria but with significant urobilinogenuria, probably as a result of increased red cell destruction [1738]. The increase in Bromsulphalein retention is primarily related to the fever but sometimes persists after the fever subsides [1482, 1738]. Decrease of the serum cholesterol and the cholesterol ester ratio has been reported [1837]. Elevated blood-tyrosine levels have been found [641]. Cephalin flocculation and thymol turbidity are increased [1317, 1738, 1837] associated with a reduction of albumin and increases of alpha₁, beta, and gamma globulins [3307]. In naturally occurring malaria, abnormal results of cephalin flocculation, colloidal gold, and thymol-turbidity tests, with increased serum bilirubin and Bromsulphalein retention, have been reported [506, 1720, 2024, 2061, 2121, 2132, 3586].

Structural Alterations. Liver biopsy specimens in the nonfatal, naturally occurring form show variable changes [660, 2152, 3311, 3586]. In some, the livers are normal, but in the majority, nonspecific reactive hepatitis is noted, with swollen hepatic cells, many mitotic figures, and proliferated Kupffer cells. In addition, acidophilic hyalinization of an occasional cell is seen. In a few cases in which the general reaction is more severe, focal necrosis and even granulomas are found [3311]. Portal inflammation is usually present. The increased mitoses persist for a considerable time, being found in biopsies after the disease has subsided [660]. In acute malarial attacks, enlargement of the liver is due to increased blood content and edema. The Kupffer cells are large and bulge into the sinusoids, which they often obstruct. They frequently contain refractile, finely divided pigment granules, as well as parasites or parasitized red cells and cellular debris [75, 3159]. Little hemosiderin is noted. In fulminant malaria, many parasites are found in the red cells in the hepatic sinusoids, which are very much congested. Mononuclear cells rich in pigment and apparently desquamated Kupffer cells or other reticuloendothelial cells are intermixed. The hepatic cells are free of pigment and parasites, and occasionally they show fatty degeneration.

EXTENSIVE HEPATIC NECROSIS. Extensive central necrosis sometimes becomes almost massive in blackwater fever (characterized by severe hemolytic jaundice and toxic nephrosis) and other instances of falciparum infection [75, 1709]. This is partly the effect of shock and partly the result of obstruction of the lobular circulation by enlarged Kupffer cells, by clumped red cells [1809], or by constriction of the central vein [2176]. Central necrosis is probably an agonal change in many instances, rather than the result of an intravital process.

EXTRAVASCULAR PHASE OF MALARIA. In the intervals between attacks, the liver is not much enlarged but is gray in color. The parenchymal cells are normal. The Kupffer cells are enlarged and loaded with heavily clumped pigment, only a small

part of which is derived from the parasites. The bulk of the pigment is comprised of hemoglobin-degradation products, chiefly bilirubin and hema- tin. Some hemolysis may be present, with high indirect-reacting serum-bilirubin levels, pleiochromia of the bile, and the common occurrence of pigmented gallstones. The histiocytes in the portal tracts also contain some pigment, and some stellate fibrosis is occasionally noted.

"MALARIAL CIRRHOSIS." Repeated attacks, especially of falciparum malaria, have been claimed to cause hepatic fibrosis eventually. The existence of a malarial cirrhosis is doubtful, and in most of these instances other hepatotoxic and nutritional factors are probably responsible [109, 739, 943]. The malarial parasites in chronic falciparum infection supposedly lodge in the liver rather than in the spleen.

Leishmaniasis

Visceral leishmaniasis (kala-azar) is characterized by infestation of the entire reticuloendothelial system, including the Kupffer cells. In tissue sections the parasites resemble *Histoplasma capsulatum* (see Histoplasmosis, Chap. 54). In the liver, the enlargement of the Kupffer cells leads to disturbances of the hepatic blood flow, with enlargement of the entire organ. In addition, the Kupffer cells contain hemosiderin, and the hepatic cells are atrophic, owing to compression. In severe cases, the flagellates are found in the hepatic cells, resulting in degenerative changes of these cells, even in biopsy specimens [2976]. Macrophages in the portal tracts also contain parasites. Incidental nonspecific alterations include fatty changes, edema, and even central necrosis. In keeping with the proliferation of the Kupffer cells and other reticuloendothelial^o cells, the serum-gamma globulin level is very high and the results of the flocculation tests are abnormal [2363].

Trypanosomiasis

In American and African trypanosomiasis, enlargement of the liver occurs with areas of focal necrosis and fatty infiltration.

DIFFUSE HEPATIC DISEASES WITH CHOLESTASIS OF UNKNOWN ETIOLOGY

In the diseases of this group a viral or toxic etiology is suspected in some instances, whereas in others no etiologic factor is known. Intrahepatic cholestasis is common to most of them. The clinical features, laboratory findings, and even the histologic changes in the liver frequently resemble those seen in extrahepatic biliary obstruction.

Classification

Neonatal, or giant-cell, hepatitis
Cholangiolitis and pericholangiolitis
Hepatitis in adolescent girls

Giant-cell Hepatitis

A form of hepatitis morphologically characterized by hepatocellular giant cells and evidence of cholestasis is gradually being separated from the large group of neonatal jaundices [679, 784, 1394, 2960, 3234] (see Neonatal Jaundice, under Proposed Classification of Jaundice, and Jaundice in the Neonatal Period, under Discussion of Proposed Classification, Chap. 21). This giant-cell hepatitis also includes most instances of hepatitis which occur in the first years of life. The etiology of the disease is not established. The biliary passages are grossly and microscopically patent. No evidence can be found for congenital syphilis, with which such cases were formerly associated. Rh incompatibility usually can not be demonstrated, but many children with this condition have various types of hemolytic anemia [1394]. Nevertheless the possibility of isoimmunization has been claimed on the basis of iron deposition, hyperplasia, and hypertrophy of pancreatic islands and kernicterus [897]. A viral etiology has been suggested for this condition, with transplacental transmission of serum hepatitis virus, although the mothers are usually asymptomatic [311, 679, 860].

In the neonatal period a viral etiology is suggested by the occurrence in siblings [784]. The etiology later in infancy is questionable. The formation of the not-very-large hepatocellular giant cells may be only a reflection of the great regenerative ability in infancy, rather than a sign of a specific disease. The functional changes may also be a reflection of youth. The prognosis is worse than that of the usual viral hepatitis in children.

Clinical Manifestations. In the neonatal period, severe jaundice may occur within the first few days of life, usually earlier than in biliary atresia, and may last several weeks or months, until either recovery or death occurs. Some infants who die within the first week of life may not be jaundiced [784]. The reported mortality is high, but since the diagnosis is made only at autopsy or biopsy, the incidence of milder cases is not established. The child eats poorly. The abdomen is distended, the liver is large, and the spleen is often palpable. Hemorrhagic tendencies and anemia are common. The stools are often acholic, and no urobilinogen is present in the urine. The tests indicative of hepatocellular damage show erratic results. The cephalin flocculation is occasionally abnormal, although serum-gamma globulin level is low. The serum-alkaline phosphatase activity is much higher than is compatible with the age. The serum-bilirubin level is high, and much of the bilirubin is prompt-reacting.

Structural Alterations. The liver is green and relatively soft. Inspissated bile is found in the extrahepatic ducts, which has led to the term "inspissated bile syndrome" (see "Inspissated Bile Syndrome," under Extrahepatic Bile Ducts, Chap. 20). Since the bile ducts are not dilated, the inspissated bile probably does not cause biliary obstruction. The histologic findings vary in such

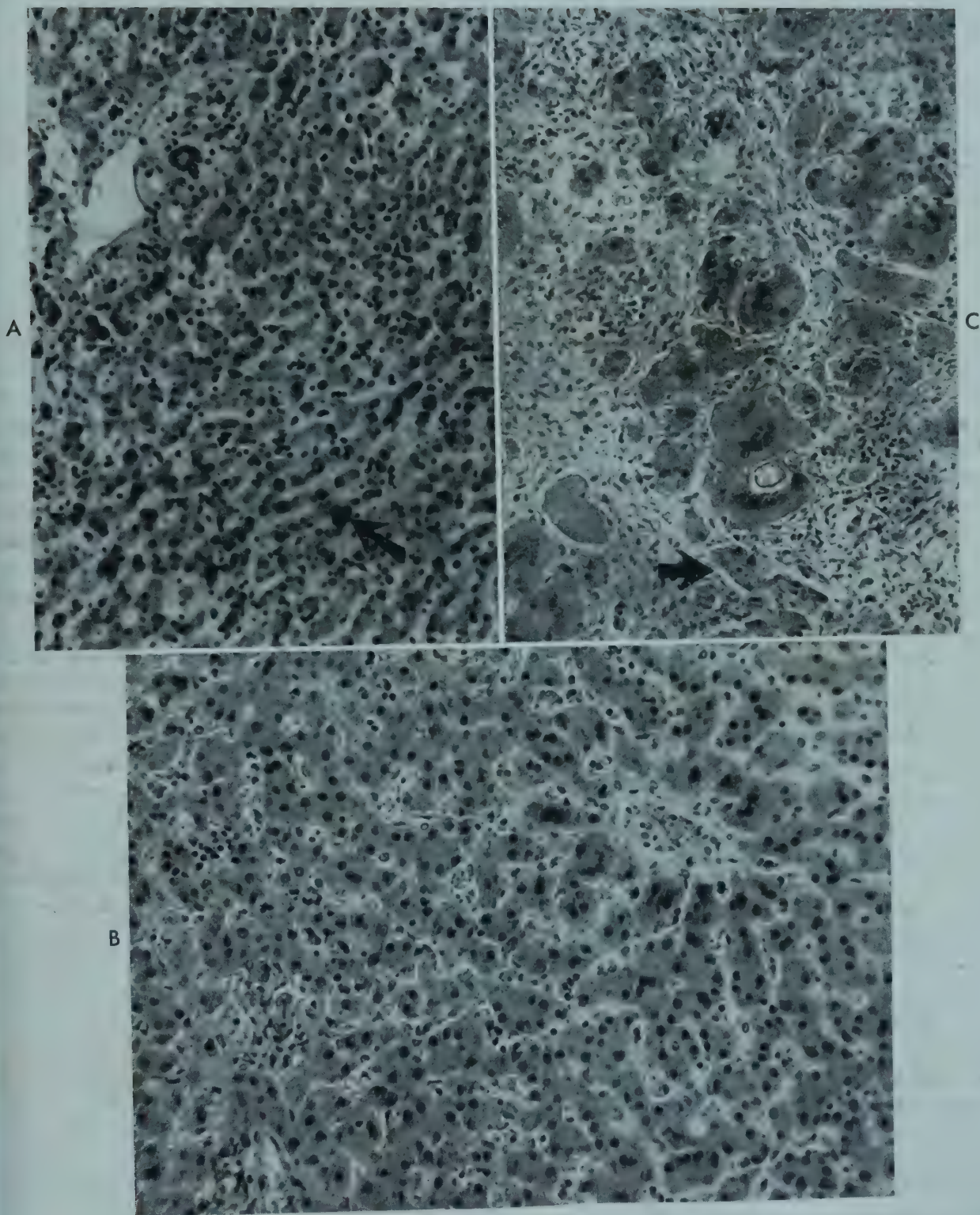


FIG. 150 Autopsy specimens of infants. H&E. A. Jaundice with intrahepatic cholestasis in the first month of life. The hepatic-cell plates are intact, and many bile casts are noted ($\times 125$). B. Conspicuous periportal regeneration with giant-cell formation in hepatitis, probably of viral origin ($\times 140$). C. Severe giant-cell hepatitis. In the collapsed and infiltrated hepatic tissue, syncytial giant cells are noted, showing in places transition into ductules (arrow). The giant cells contain vacuolated inclusions ($\times 105$).

instances [679, 860]. In some instances only centrally accentuated cholestasis, increase in intra-lobular ductules, Kupffer cell proliferation, and hematopoietic foci are found (Fig. 150A). In other instances, in addition, hepatic-cell degeneration, with zonal and occasionally central necrosis and central accumulation of balloon cells and giant cells, is associated with portal and periportal inflammation (Fig. 150B). In still other instances, the hepatic-cell plates are disrupted, and individual cells, most of which are multinucleated giant cells rich in vacuoles, iron, and bile pigment, are surrounded by collagenous membranes, giving the picture of interstitial hepatitis previously thought characteristic of congenital syphilis. Eventually the lobular architecture is destroyed, as in postnecrotic cirrhosis, except for the presence of bizarre syncytial giant cells (Fig. 150C). The histologic findings do not resemble those of viral hepatitis in adults or any other disease, although giant cells may be found in viral hepatitis as well as in congenital syphilis and in some drug reactions [1991]. Kernicterus is rare, in contrast to erythroblastosis fetalis, possibly because the jaundice is less severe and most of the bilirubin is prompt-reacting.

CHOLANGIOLITIS AND PERICHOANGIOLITIS

The diseases of this group are characterized by the predominance of intrahepatic cholestasis, a feature found in many hepatic diseases as a contributing component to other features, such as hepatic-cell damage. All clinical, functional, and structural alterations can be explained as a result of intrahepatic cholestasis alone. In some instances toxic or sensitizing agents, such as arsenicals, chlorpromazine, or methyltestosterone, have been recognized as the etiologic factors (see Allergic Cholangiolitis, Chap. 41). In other instances, the lesion possibly is a form of viral hepatitis without hepatic-cell damage from the onset, or after subsidence of hepatic-cell damage [3510]. This last form may occur in elderly people [786]. In most cases the etiology is unknown [1801, 1903, 3227]. The functional evidence of intrahepatic cholestasis in the acute stage is not associated with significant anatomic alteration, whereas in the subacute and chronic stages, alterations of the perilobular and intralobular ductules are associated with inflammation and scarring, justifying the terms "cholangiolitis" and "pericholangiolitis." The lesion is

probably a primary disorder of the ductules, or "cholangiolitis," with secondary involvement of the surrounding area as a result of the escape of bile through the ductules [2767, 3510], rather than a primary inflammation around the ductules with secondary encroachment of them, or "pericholangiolitis" [2153, 2156] (see Pericholangiolitis, under Focal Necrosis, Chap. 25).

Acute Cholangiolitis

Moderate to severe jaundice with acholic stools and pruritus but with few systemic manifestations characterizes acute cholangiolitis. This stage is not clearly differentiated from other hepatic disorders but becomes so if it progresses into the subacute stage, although the lesion may subside completely within 3 or 4 weeks. The liver is neither much enlarged nor tender. The laboratory findings indicate cholestasis with few if any signs of hepatic-cell degeneration. The results of the flocculation tests are normal. Urobilinogen is not necessarily absent from the urine, and the serum alkaline phosphatase is more often increased than is the serum cholesterol. In liver biopsy specimens, bile stasis is noted in the center of the lobules. The hepatic cells are otherwise unchanged. The portal tracts often contain inflammatory cells, including segmented leukocytes, while the ductules do not appear altered (Fig. 97A and B).

Subacute Cholangiolitis

Subacute cholangiolitis is characterized by jaundice of several months' duration and is a well-substantiated clinical and pathologic entity. It may represent the initial stage of chronic cholangiolitis, with its relentless advance, or the clinical manifestations including jaundice may subside after several months without any obvious clinical sequelae. In some instances cirrhosis possibly develops insidiously after a latent period [3510].

Clinical Manifestations. The signs and symptoms resemble those of extrahepatic biliary obstruction, with severe itching and some nausea but without pain and with less systemic reaction than in viral hepatitis with an equal degree of jaundice. The liver is large, smooth, and nontender, and the spleen is not palpable. The laboratory findings indicate cholestasis. The total serum-cholesterol level is occasionally very high [3236]. Urobilinogen may be absent from the urine for long periods and the stools are often acholic. Some groups have recently recommended two procedures to differ

entiate intrahepatic from extrahepatic cholestasis. One is laparoscopy with percutaneous cholecystocholangiography, which reveals patent and undilated bile ducts. The other is the administration of cortisone, which causes a drop in the serum-bilirubin level in intrahepatic cholestasis.

Structural Changes. Hepatic-cell degeneration is not prominent in the majority of cases, but rarefaction and acidophilic degeneration of the cytoplasm may develop. Severe bile stasis is present in the center of the lobules, and bile plugs are noted in the distended bile canaliculi. In contrast to the acute stage, portal and periportal inflammation, with mononuclear cells and a variable sprinkling of segmented leukocytes, is conspicuous (Fig. 98). This infiltration extends into the periphery of the lobules around proliferated perilobular ductules. The ductular epithelium is proliferated segmentally, with sloughing of the epithelial cells and exudate in the lumen and between the epithelial cells and the basement membrane. The liver contains bile plugs, and the periductular tissues show varying degrees of inflammation. The inflammatory reaction is not concentrically arranged around the portal tracts and sometimes extends deep into the lobule. The portal tracts become stellate, and perilobular fibrosis develops in cases of longer duration. The larger bile ducts, as well as the extrahepatic biliary tree, are normal. The inflammatory changes associated with subacute intrahepatic cholestasis are not diagnostic histologically, since they occur without cholestasis and without the corresponding clinical and functional manifestations.

Rapidly Fatal Cholangiolitis

Rarely, cholangiolitis causes a rapidly developing disease with a fatal outcome within a few months [2758]. It begins with severe jaundice, weight loss, pruritus, and hepatomegaly. The liver is enlarged and appears smooth or slightly nodular. Intrahepatic cholestasis predominates in the laboratory findings. Structurally the signs of severe intrahepatic cholestasis are associated with those of submassive necrosis. The lesion appears to be a combination of hepatocellular and ductular involvement. Severe inflammation of the portal tracts is present, with excessive proliferation of the ductules. They contain bile plugs and are surrounded by acute and chronic inflammatory exudate and recent fibrosis. These changes extend in fingerlike streaks into the lobular parenchyma and occasion-

ally occupy the entire lobule. This is associated with bile stasis, degeneration of hepatic cells, and submassive necrosis (Fig. 151, upper).

Chronic Cholangiolitis and Cholangiolitic Cirrhosis (Primary Biliary Cirrhosis)

Despite its relative rarity, chronic cholangiolitis is a disease with many names. These include "hypertrophic cirrhosis of Hanot" [1696], "primary biliary cirrhosis" [27], "cholangiolitic cirrhosis" [2797, 3510], "chronic obliterating cholangitis" [1801], and "pericholangiolitic biliary cirrhosis" [2156]. The alteration of lipid metabolism in this disease, resulting in hypercholesteremia, may be so severe that xanthomas develop and even dominate the clinical picture (xanthomatous biliary cirrhosis). Otherwise this form seems to have the same clinical, functional, and structural manifestations and the same course as chronic cholangiolitis without cutaneous xanthomatosis [27, 2156].

Chronic cholangiolitis is characterized by a protracted clinical course with severe jaundice, without ascites, portal hypertension, or signs of hepatocellular insufficiency. The jaundice interferes surprisingly little with the condition of the patient. Appetite and nutrition remain unimpaired. Chronic cholangiolitis itself is therefore not fatal; but neither is it amenable to other than symptomatic treatment. After a long period, usually 2 to 12 years, septal cirrhosis becomes superimposed on the cholangiolitic pseudocirrhosis (see Periductular Fibrosis; Pseudocirrhosis, Chap. 28). Then manifestations of portal hypertension and hepatocellular insufficiency with ascites develop, and often a rapid downhill course, characteristic of any type of cirrhosis, leads to death.

Clinical Manifestations. The disease occurs mainly in women in the later child-bearing period and after the menopause. Jaundice begins insidiously and lasts many years, with some fluctuation in almost all cases [27, 2156, 2767, 3510]. It is usually associated with severe pruritus, which often dominates the picture and precedes the jaundice by more than a year. Manifestations pointing to hepatocellular damage, such as spider nevi, severe malaise, fat intolerance, nausea, or vomiting, are usually absent for many years, as are abdominal pains, chills, or fever, which one would expect in biliary tract diseases. Diarrhea with steatorrhea and hemorrhagic tendencies caused by decreased vitamin K absorption occur occasionally

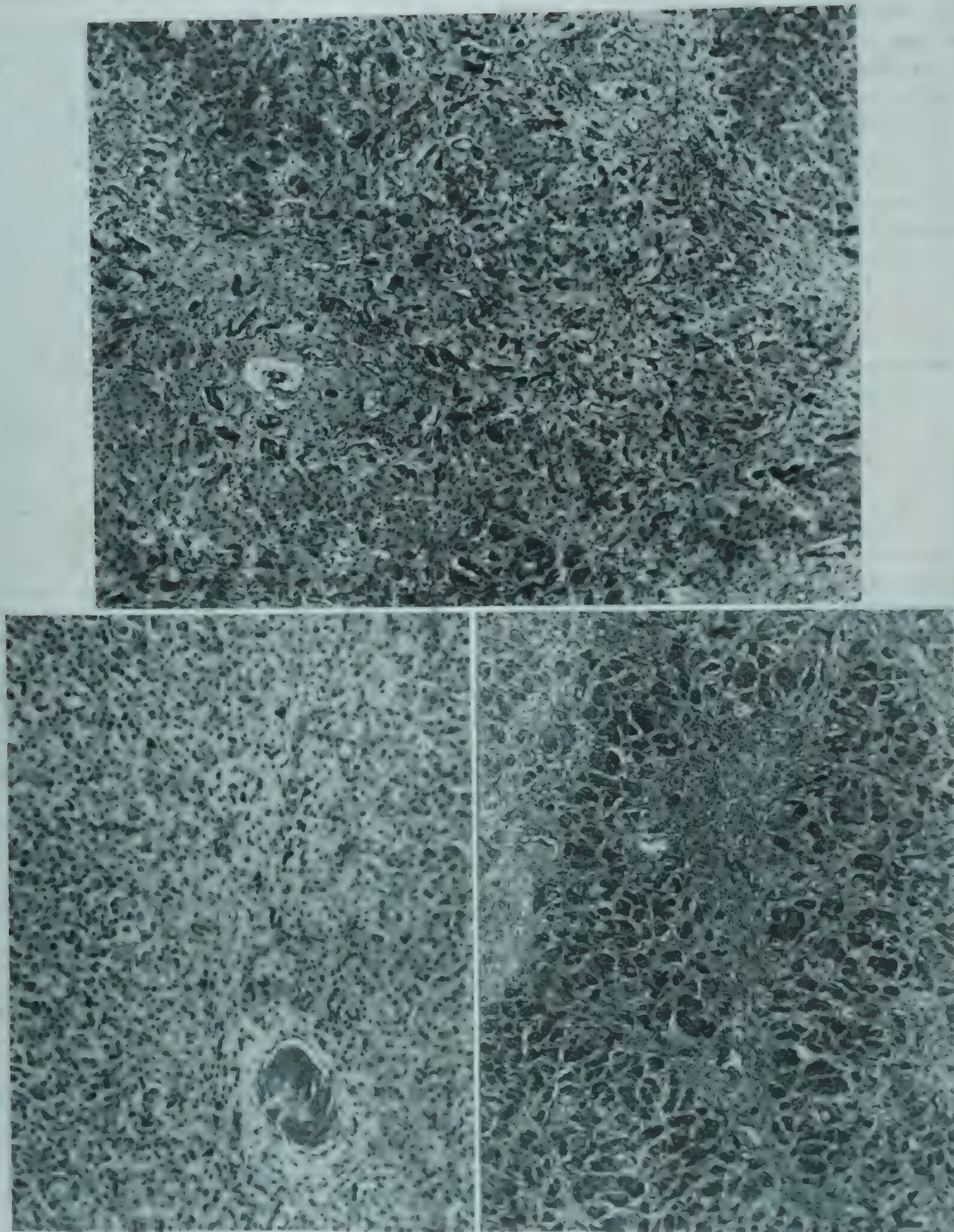


FIG. 151 Autopsy specimens. H&E. *Upper.* Acute fatal cholangiolitis. Proliferation of ductules, surrounded by inflammatory exudate and submassive necrosis of parenchyma ($\times 60$). *Lower left.* Chronic cholangiolitis (without dermal xanthomas), showing periportal fibrosis and some bile duct proliferation, while the hepatic cells show only slight changes ($\times 120$). *Lower right.* Cholangiolitic cirrhosis. Hepatic cells and hepatic-cell groups are separated by connective tissue fibers. Beginning formation of septums ($\times 70$).

[926]. The liver is very much enlarged, smooth, and nontender. The spleen is enlarged. Skin changes include hyperpigmentation with increased melanin to such a degree that cases have been considered to be hemochromatosis [482]. Dermatoses are often present as a result of the itching and possible vitamin A deficiency and also as a direct expression of the disease. Lymphadenopathy is frequently found [27]. Osteoporosis with compression fractures of the spine is observed in protracted cases [1080, 1903], and clubbing of the fingers is usually present. In late stages, the picture does not differ from Laennec's cirrhosis with severe jaundice. Esophageal varices with hematemesis and spider nevi develop. Eventually hepatic coma with severe bleeding tendencies ushers in the final stage.

Laboratory Findings. The urine is dark and rich in bilirubin, and it is seldom free of urobilinogen. The stools are light, but rarely clay-colored. Fecal urobilinogen is reduced but not absent. Fecal fat is increased, and fat absorption is impaired. Moderate anemia is present, and the white blood count is normal. The serum albumin is somewhat decreased, but the globulins are greatly increased, especially the α_2 , β , and γ fractions. The flocculation tests show inconsistent results, except for thymol turbidity, which is increased. Total serum-cholesterol and phospholipid levels are increased, and the cholesterol/ester ratio is moderately reduced. Serum-vitamin A level is low and Bromsulphalein retention is increased. Prothrombin time is usually normal, or if it is prolonged, it responds to vitamin K administration. The basal metabolic rate is elevated without evidence of hyperthyroidism [27, 2156, 3510]. In late stages, all laboratory manifestations of diffuse septal cirrhosis develop.

Structural Alterations. The earlier stages, not yet complicated or obscured by septal cirrhosis, are known from biopsy specimens [27, 2156]. They show severe portal and periportal inflammation and bile stasis with ductular proliferation similar to that in the subacute stage, except that fibrosis is more prominent (Figs. 151, lower left, 152, upper right). The limiting plate is destroyed, and fibrosing inflammation extends, without sharp limitations, into the parenchyma, especially along the perilobular and intralobular ductules. The portal tracts enlarge and become stellate. Perilobular fibrosis develops, and hepatic cells or small groups of them appear separated by interstitial mononuclear infiltration and fibrosis without the lobular

pattern being destroyed. Grossly the liver is larger than normal, firm, and dark green (or brown in instances with little jaundice). The surface is smooth to finely granular with only a few uneven depressions. The lobular architecture can not be discerned. The biliary passages are usually entirely normal. Increase of connective tissue, with collagenous fibers between the hepatic-cell plates, gives the impression of cirrhosis, although the formation of regenerative nodules and septums may still be in the background (Fig. 151, lower right). In later stages the ductules and even the smallest interlobular bile ducts are reduced in number, apparently destroyed by the fibrosing inflammation [2156] (Fig. 152, lower right). The hepatic cells are fairly well preserved, except for some focal feathery degeneration and some necrosis and disappearance near the periportal inflammation. Eventually the periportal inflammation extends to the central vein in places, and septums dissecting the lobules, as well as regenerative nodules, develop [2359]. The lobular architecture becomes distorted (Fig. 152, upper left). Hepatic-cell damage becomes severe, presumably because of the altered circulation. At the time of death, cirrhosis is usually far advanced, so that the picture is almost indistinguishable from that of diffuse septal cirrhosis, as far as connective tissue distribution and hepatic necrosis are concerned.

"Xanthomatous Form" of Cholangiolitic Cirrhosis. Chronic cholangiolitis is sometimes associated with disturbances in lipid metabolism resulting in deposition of lipids in the skin [27, 256, 2153, 2156, 2300, 2767]. These yellowish infiltrations appear first in the eyelids (xanthelasma) and subsequently elsewhere in the skin as raised yellow plaques. Flat xanthomas are observed on the palms, neck, chest, and back, while tuberous xanthomas develop over finger joints, wrists, elbows, ankles, and the Achilles tendon, as well as over pressure points and scars. They appear months to years after the onset of the disease. They are not found in internal organs and do not cause biliary obstruction, as previously assumed [27, 3317], but are rather a consequence of the disease. Nevertheless the skin changes have given rise to the commonly used term "xanthomatous biliary cirrhosis." The serum-cholesterol level is greatly elevated, and levels over 2,000 mg per 100 ml have been recorded. The cholesterol/ester ratio is usually lowered. The phospholipids, chiefly lecithin, are much increased, reaching levels above

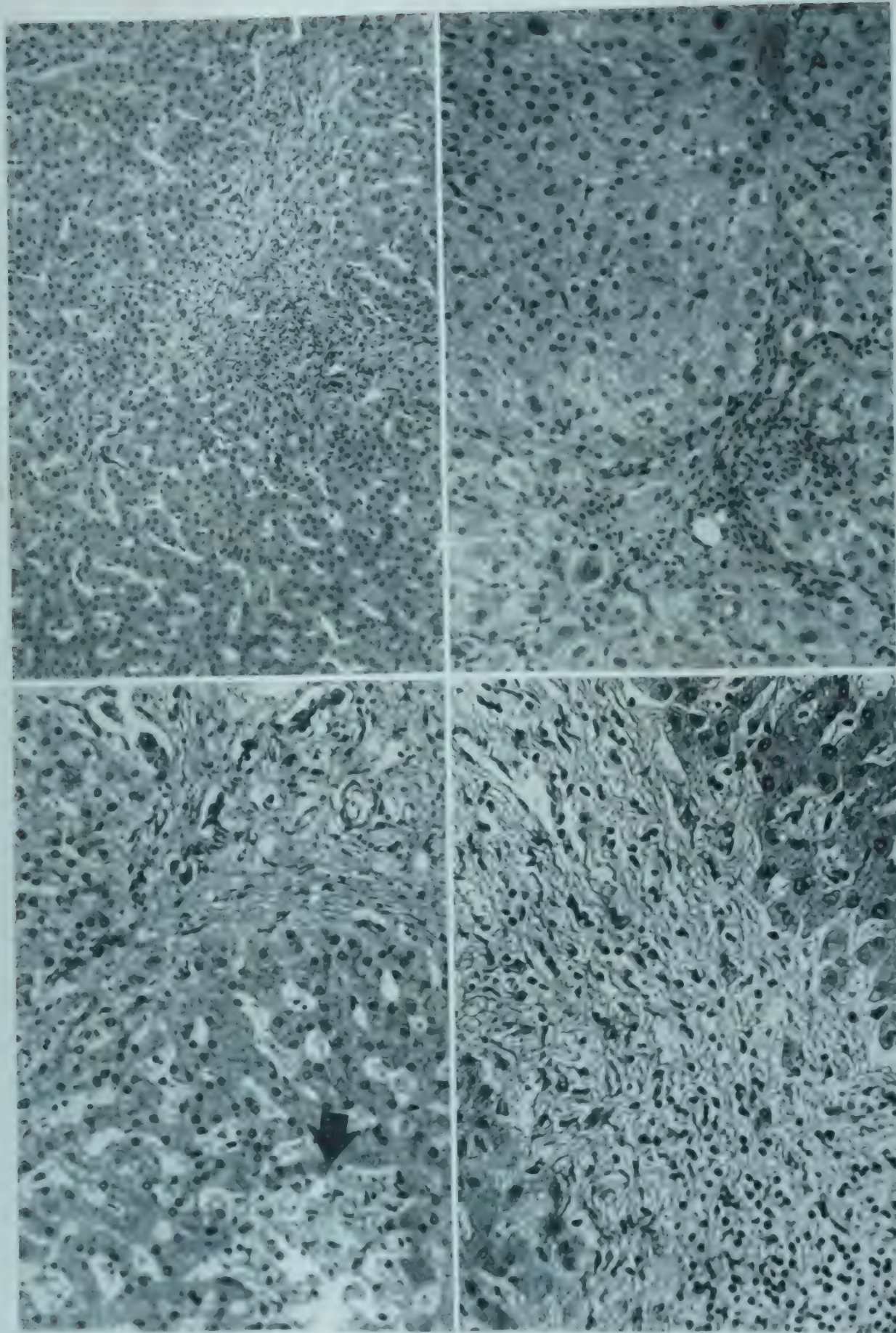


FIG. 152 *Upper left.* Autopsy specimen of patient subsequently dying from cholangiolitic cirrhosis with dermal collagenosis ("xanthomatous biliary cirrhosis"). Note portal and periportal fibrosis and inflammation, with beginning distortion of the lobular patterns. H&E ($\times 120$). *Upper right.* Autopsy specimen of same patient as in upper left. Note portal and periportal infiltration. H&E ($\times 180$).

80 mg phospholipid phosphorus per 100 ml. The amount of neutral fat is normal according to some [2156] and elevated according to others [27]. Beta globulins are increased. Despite the high total fat content, the serum is usually not lipemic, and atherosclerosis is not more severe than in normal persons. With moderate elevation of the total lipids, xanthelasmas appear; with an increase of the total lipids above 1,800 mg per 100 ml generalized xanthomas appear. In the later stages of the disease, when diffuse septal cirrhosis with hepatocellular failure develops, the lipid levels drop and the xanthomas disappear [26]. Complete recovery with improvement in liver function is apparently possible [1414].

DIFFERENTIAL DIAGNOSIS OF CHOLANGIOLITIC CIRRHOSIS WITH XANTHOMAS. Cholangiolitic cirrhosis with xanthomatosis is a primary nonfamilial liver disease with no xanthomas in the liver. It should be differentiated from (1) familial hypercholesteremic essential xanthomatosis [3317], in which the degree of liver involvement, if present at all, is clinically and histologically insignificant; (2) the secondary lipemias, such as those seen in diabetes mellitus, in which the liver enlarges because of fat accumulation; (3) phosphatide-storage (or Niemann-Pick) disease or cerebroside-storage (or Gaucher's) disease, in which the liver is enlarged as part of generalized involvement of the reticuloendothelial system (see Lipid-storage Diseases, Chap. 53); (4) lipid granulomatosis (or Hand-Schüller-Christian disease), in which granulomas appear in the liver with deposition of lipids,

particularly cholesterol in the absence of hypercholesteremia [2274]. Liver function is not impaired in lipid granulomatosis, and jaundice, if present, is caused by extrahepatic obstruction of the ducts by lymph nodes.

Treatment. In the acute and subacute stages, the differentiation from extrahepatic biliary obstruction is important, to forestall superfluous surgery, which is poorly tolerated. In any stage the patient should be thoroughly questioned as to exposure to drugs which initiate allergic cholangiolitis. ACTH or cortisone has been recommended in the treatment of the disease; in some instances they have caused a drop in the serum-alkaline phosphatase and cholesterol levels. Methyltestosterone has been used to control pruritus, although it may raise the serum-bilirubin level. Supplemental calcium and vitamin D should also be given because of the frequently associated generalized osteoporosis.

Hepatitis in Adolescent Girls

A protracted form of jaundice may rarely develop in young girls when they are near puberty. This begins like viral hepatitis with enlargement of the liver and spleen, but eventually the phenomena associated with cholangiolitis predominate. Clubbing of the fingers is conspicuous, and pulmonary fibrosis is observed in some cases. The serum-gamma globulin level is high. In those who die, a cholangiolitic cirrhosis is found. The etiology of this rare and not clearly defined condition is obscure.

Lower left. Autopsy specimen from child with biliary atresia. Note proliferation of ductules surrounded by fibrosis and small bile infarct in right lower corner (arrow). H&E ($\times 150$). *Lower right.* Same as upper left. Note portal and periportal fibrosis and inflammation and absence of ductules. H&E ($\times 150$).

HEPATIC INJURY FROM EXTRAHEPATIC BILIARY OBSTRUCTION

Extrahepatic biliary obstruction produces hepatic damage. This was originally considered a late sequel of obstruction, but results of more recent hepatic tests and liver biopsies revealed that such changes occur early. They are not a primary disease entity and are usually described as a consequence of extrahepatic biliary obstruction, or cholestasis (see Extrahepatic Biliary Obstruction, Chap. 24). The earliest change is simple cholestasis devoid of any inflammation. The reaction to cholestasis, which includes portal and intralobular inflammation, must be considered an entity, for differential diagnostic purposes. The term "biliary hepatitis" appears to be the most appropriate, since it emphasizes the diffuse hepatic lesion, with an inflammatory component, and the necessity of considering this lesion a hepatic injury for purposes of clinical management. Since bacterial infection frequently complicates extrahepatic biliary obstruction and alters the clinical and laboratory findings considerably, noninfected biliary hepatitis is separated from infected biliary hepatitis. Most of the features have been discussed in the chapter on cholestasis (see Extrahepatic Biliary Obstruction, Chap. 24); the following is a review mainly for purposes of clinical classification.

Classification

Simple cholestasis

Biliary hepatitis

Biliary fibrosis

Infected biliary hepatitis

Intrahepatic cholangitis (see Intrahepatic Purulent Cholangitis, Chap. 25)

Extrahepatic cholangitis (see Extrahepatic Cholangitis, Chap. 25)

Multiple cholangitic abscesses (see Cholangitic Abscesses, Chap. 56)

Secondary biliary cirrhosis

Simple Cholestasis

The hepatic alteration resulting from biliary obstruction of short duration is simple cholestasis. Bile pigment is seen in the Kupffer cells and in the bile canaliculi in the form of casts in the center of the lobule. The hepatic cells show some bile pigment granules and a moderate amount of atrophy (Fig. 95A and B).

Prompt-reacting bilirubin in serum is increased, as is the serum alkaline phosphatase, and bilirubin is present in the urine. Tests indicating hepatocellular degeneration show normal results. The serum proteins and other serum enzymes are normal. Bromsulphalein retention is increased.

The chief clinical manifestations of simple cholestasis are jaundice, pruritus, and mild anorexia. Malaise is not a feature of early stages, in contrast to the condition in jaundice from hepatic-cell degeneration. The liver is slightly enlarged and not tender, while the spleen is small. The absence of pain suggests malignancy, or "silent obstruction," although this also occurs in intrahepatic cholestasis, or cholangiolitis, and occasionally in the presence of biliary calculi. In malignant obstruction, the gallbladder is often enlarged and palpable, while in the presence of stones it is small because of the associated inflammation with scarring. (Courvoisier's law). The symptomatology may be modified by (1) hepatic-cell damage, which produces severe anorexia and malaise; (2) progression of a malignant process, which produces weight loss, back pain, and malaise; (3) biliary dyskinesia and spasm of the sphincter of Oddi.

usually owing to a stone in the ducts, which produces colic, nausea, and vomiting; (4) bacterial infection, producing chills and fever (see Bacterial Infection, under Extrahepatic Biliary Obstruction, Chap. 24; also Infected Biliary Hepatitis, later in this chapter).

Biliary Hepatitis

The duration of cholestasis required to produce hepatic-cell damage and the mesenchymal reaction in the form of biliary hepatitis depends on the degree of obstruction and on the criteria used to define the lesion. In cases subjected to biopsy, biliary hepatitis is far more common than simple cholestasis [1934]. Complete obstruction, often malignant in nature, produces biliary hepatitis more rapidly than incomplete obstruction typically produced by calculi. Strictures are usually associated with biliary hepatitis. Hepatic changes other than those of bile imbibition occur after 1 week of complete obstruction or 2 weeks of incomplete obstruction [2802] (Figs. 96, 153, upper left) (see Extrahepatic Biliary Obstruction, Chap. 24). Several causes for the liver damage have to be considered: (1) protoplasmic injury by retained biliary substances; (2) compression of the hepatic cells by the bile stasis; (3) swelling of the hepatic cells interfering with blood flow [1497]; (4) interference with blood flow owing to compression by the dilated intrahepatic bile ducts [674, 2202]. The lesion can be considered comparable to that of toxic hepatitis. Grossly, the liver is enlarged and dark green. The lobular architecture is conspicuous, the dark central zones contrasting with a light-green periphery. The intrahepatic bile ducts are dilated.

While functional alterations referable to cholestasis, such as hyperbilirubinemia, elevated serum-alkaline phosphatase activity and total cholesterol, and absence of urinary urobilinogen, occur rapidly, those referable to hepatocellular degeneration appear after 1 week. A drop in serum-cholinesterase level and decreased hippuric acid synthesis occur early, while serum albumin and cholesterol esters decrease somewhat later. Galactose tolerance is impaired only after a month or longer. Functional evidence of mesenchymal irritation, such as gamma globulin elevation, appears late, if at all, in the absence of infection. Because of this and because of an unidentified factor which depresses flocculations, the serum-protein reactions, such as the cephalin flocculation or thymol turbidity, remain normal even in the presence of

significant hepatic-cell damage and inflammation.

The clinical features of biliary hepatitis are similar to those of simple cholestasis. The liver is definitely enlarged and tender, especially in the gallbladder region. The spleen is not palpable. If biliary hepatitis is protracted, hemorrhagic tendencies develop which can be relieved by administration of synthetic vitamin K salts parenterally. Biliary hepatitis is usually fatal in 6 to 9 months if the obstruction is not relieved. In acute biliary hepatitis, the laboratory evidence of cholestasis outweighs the indications of hepatic-cell degeneration [2277, 2641].

Chronic Biliary Hepatitis (Biliary Fibrosis)

The late result of noninfected biliary hepatitis is hepatic fibrosis, often called "cholestatic cirrhosis" [2797], "obstructive cirrhosis" [2154], or "biliary cirrhosis" [1696, 2359], although it rarely fulfills the criteria for cirrhosis. Of the terms mentioned, the last is the best. Grossly the liver is firmer than in biliary hepatitis. The surface is smooth, and the lobular architecture is slightly obscured, the portal tracts being white and prominent. Microscopically the portal tracts are enlarged and stellate. Fibrosis develops around proliferated intralobular and perilobular ductules. Regenerative nodules and septums stimulated by inflammation in the portal tracts are only exceptionally seen (Fig. 153, upper right). In very rare instances diffuse septal cirrhosis eventually results. In animals, particularly rabbits, such a biliary cirrhosis rapidly develops. Biliary cirrhosis has been found in children with biliary atresia who tolerate complete cholestasis for years [865] (Fig. 152, lower left) (see Atresia, under Biliary System, Chap. 20). The existence of noninfected biliary cirrhosis in adults has been denied by some [943], but maintained by others [1696, 2359], since complete obstruction does not often permit survival for a sufficient length of time for development of the condition and since the formation of regenerative nodules is suppressed in the presence of complete obstruction.

Cirrhosis occurs in the very exceptional case of extremely prolonged incomplete obstruction without infection.

The laboratory findings are similar to those for biliary hepatitis, except that the cholesterol/ester ratio is usually reduced, the total serum-protein level is low, as are the levels of serum albumin and gamma globulin, although α_2 and beta globulin levels may be elevated. Cephalin flocculation

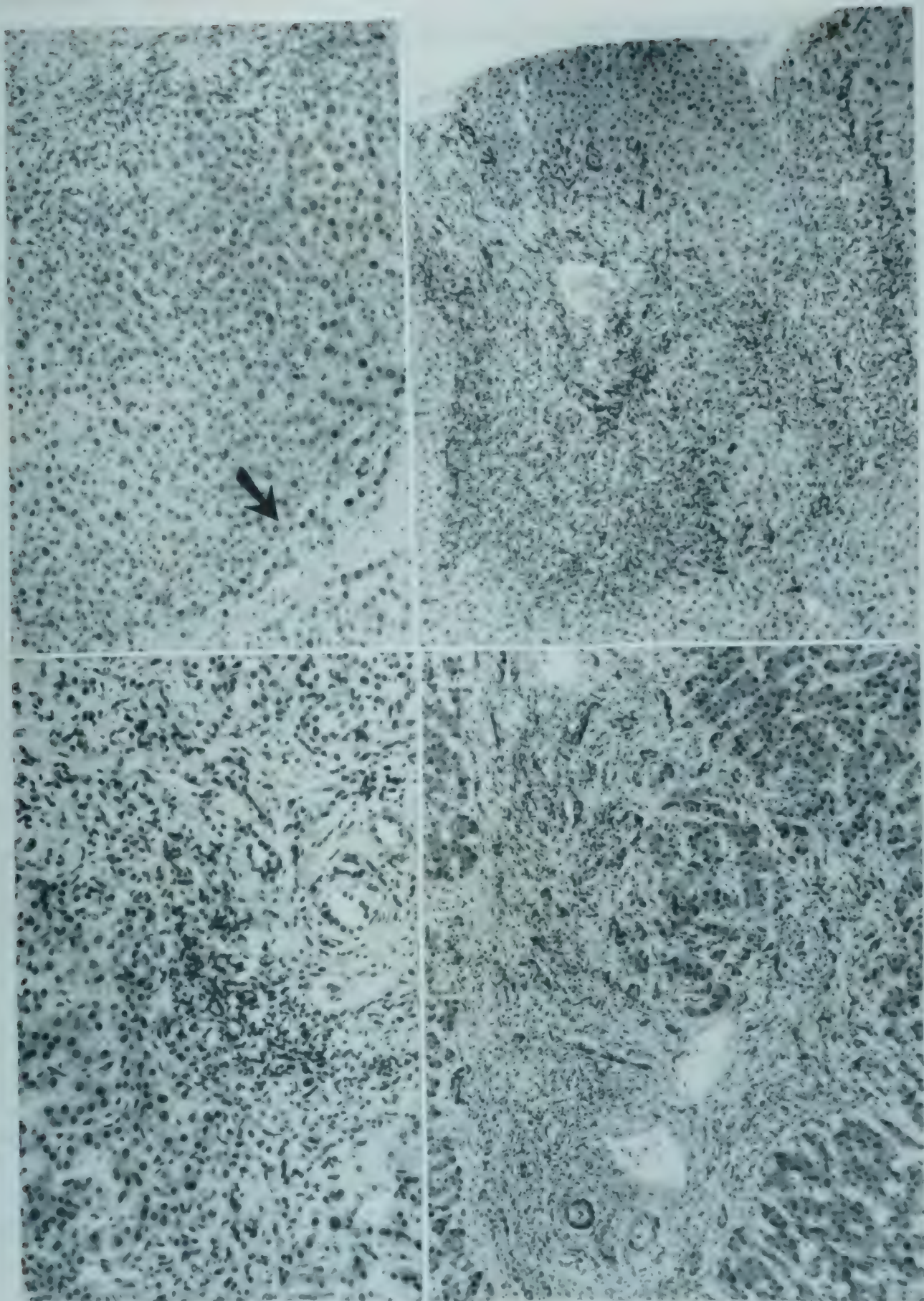


FIG. 153 *Upper left.* Biopsy specimen of biliary hepatitis without infection after extrahepatic biliary obstruction of 2 weeks' duration. Severe central bile stasis (arrow) is present, with little inflammatory exudate in the portal tracts. H&E ($\times 95$). *Upper right.* Biopsy specimen of beginning cholangitic cirrhosis caused by common duct stricture. Note portal and periportal exudate and beginning destruction of the lobular architecture. H&E ($\times 75$). *Lower left.* Biopsy specimen in infected biliary

is rarely abnormal; thymol turbidity, zinc sulfate turbidity, and the serum mucoproteins are slightly increased. The prothrombin time before administration of vitamin K is prolonged.

Severe itching and evidence of malnutrition, including osteoporosis, are usually present in protracted obstruction. The liver is enlarged, firm, and somewhat tender. The spleen is usually not palpable.

Infected Biliary Hepatitis

Bacterial infection of the liver frequently complicates extrahepatic biliary obstruction. This occurs more often in strictures and choledocholithiasis than in carcinoma [2277, 2632, 3477]. These conditions are sometimes associated with suppuration or empyema in the gallbladder or bile ducts and with purulent pyelephlebitis. The liver is invaded by pyogenic bacteria through the portal vein branches or, less often, through the bile ducts. Hepatic-cell degeneration, as well as portal inflammation with the appearance of segmented leukocytes (Fig. 153, lower left), develops in response to the bacteria themselves, or to their products, or to injurious material which has passed through the sinusoids into the tissue spaces. Hematogenous and lymphatic routes are more important than ascending infection through the bile duct in the production of infected biliary hepatitis. Hepatic injury produced does not depend so much upon the duration and degree of obstruction, as is the case with noninfected biliary hepatitis, as upon the extent of inflammation and upon factors favoring infection, such as diabetes.

Grossly the liver is the same as in biliary hepatitis, except that its consistency is reduced. Infected biliary hepatitis may eventually progress to the formation of pyelephlebitic or cholangitic abscesses (see *Pylephlebitic Abscesses*, and *Cholangitic Abscesses*, Chap. 56).

The laboratory findings of noninfected biliary hepatitis are altered by reduction of serum albumin and of cholesterol/ester ratio. Cephalin flocculation becomes abnormal, as do thymol turbidity and zinc sulfate turbidity [2277]. These abnormalities of the serum protein reactions have been explained by excessive gamma globulin formation

caused by the inflammation despite the low total serum protein. This formation does not necessarily take place in the liver. Therefore a superimposed bacterial infection transforms the laboratory findings of a biliary hepatitis into those of a primary hepatitis, for instance of viral etiology, and thus obscures the laboratory differences between surgical and medical jaundice.

The clinical manifestations of infected biliary hepatitis are chills, fever, leukocytosis, and anemia, in addition to those of noninfected biliary hepatitis. Intermittent fever (Charcot's fever) may be present, especially if cholangitis temporarily blocks part of the intrahepatic biliary tract (see *Intrahepatic Purulent Cholangitis*, Chap. 25). The liver is enlarged and tender.

Purulent Biliary Hepatitis. Chronic extrahepatic biliary obstruction with mild jaundice and slight biliary hepatitis may be complicated by a sudden bacterial infection that differs from the infected biliary hepatitis mainly in its virulence. The routes of infection are the same, but in purulent biliary hepatitis, suppurative thrombosis of the portal vein or its branches is a common aggravating factor and abscesses are frequently found in the liver. The name "purulent hepatitis" is proposed for this disease, which has differential diagnostic significance because of its dramatic onset and because it resembles acute hepatic insufficiency from massive necrotic hepatitis or cirrhosis. Jaundice, which was mild or absent, suddenly deepens, the total bilirubin level rapidly exceeding 20 mg per 100 ml within 1 week. The rapid deepening of the jaundice can be explained by a combination of severe hepatic-cell damage, blockage and rupture of the ductules, and Kupffer cell-hepatic-cell block because of damage to the hepatic cells. Alkaline phosphatase activity also rises very rapidly. The liver is tender, and excursion of the right diaphragm is limited. Nausea and vomiting are prominent, and renal failure occurs, with rapidly developing azotemia. The laboratory findings are those of infected biliary hepatitis with a great increase in the serum-gamma globulin level and in the abnormal results of the turbidity tests. The condition is often fatal within 1 to 2 weeks. Evidence of septicemia, severe leukocytosis, and high serum-

hepatitis caused by stone in the common duct. Portal, periportal, and intralobular inflammatory exudate includes segmented leukocytes. H&E ($\times 110$). *Lower right.* Autopsy specimen of cholangitic cirrhosis. Portal inflammation and septums extending into the lobular parenchyma and beginning nodule formation. H&E ($\times 120$).

mucoprotein levels aid in the differentiation from viral hepatitis or cirrhosis.

Chronic Infected Biliary Hepatitis (Secondary Biliary Cirrhosis)

Infected biliary hepatitis frequently causes perilobular fibrosis and, if protracted enough, cirrhosis, for which the names "cholangitic cirrhosis" [2797], "cholangitic biliary cirrhosis" [2762], "secondary biliary cirrhosis" [27], "infected obstructive cirrhosis" [2153, 2154], and "infected biliary cirrhosis" [2359] have been proposed. The lesion is most frequently the result of strictures of the biliary tract, especially after surgical injury during cholecystectomy [518, 623, 3477].

Clinically, jaundice is mild or even absent; fever is sometimes intermittent, or Charcot-like, and sometimes absent for prolonged periods. Occasionally severe jaundice persists, with a dark-green color of the skin. Pruritus is an important symptom only in the jaundiced patient. The liver is large, firm, and tender, and the spleen is moderately enlarged and firm. In some instances, the entire clinical picture of cirrhosis, with ascites, esophageal varices, and spider nevi, is noted.

Of the laboratory findings [27, 2352, 2762], elevations of serum-alkaline phosphatase activity and gamma globulin are the most characteristic abnormalities. Total cholesterol is frequently increased and exceptionally reaches levels found in cholangiolitic cirrhosis with xanthoma formation [27]. The serum-mucoprotein level is high. The urobilinogen excretion varies, depending upon the degree of obstruction. Functional evidence of hepatocellular degeneration, including hypoprothrombinemia, varies but is usually present.

Structural Changes. Grossly the liver is slightly enlarged; the color varies from brown to green, depending upon the degree of jaundice. The capsule is usually thickened, and adhesions with the diaphragm are frequent. The surface is usually finely granular, but in places it may be coarsely granular or "hobnail" in character. On the cut surface, nodules of various sizes irregularly obscure the lobular architecture. The portal tracts are greatly enlarged, gray-white, and firm, and they appear scarred. In some instances the term "pipe-stem cirrhosis" is justified. Histologically, the lobular architecture is discernible in places; the portal tracts are stellate and frequently connected by

perilobular fibrosis. Many acute and chronic inflammatory cells, as well as proliferated ductules and small bile ducts, are found in the tracts and in the septums, which show a tendency to scarring. Severe periportal inflammation is observed [910, 2359]. In addition, heavily infiltrated septums extend into the parenchyma like fingers on a hand (Fig. 153, lower right), and in places the lobule is subdivided and portohepatic vascular shunts and regenerative nodules form. Regeneration is subdued in comparison to that in other forms of cirrhosis, probably because of the presence of cholestasis. In some instances the findings are finally almost indistinguishable from those for diffuse septal cirrhosis [910], except possibly for the presence of excessive biliary pigment and conspicuous ductal as well as ductular proliferation and somewhat suppressed regeneration.

Types of Cirrhosis Related to Cholestasis

Several forms of cirrhosis are related to cholestasis, and all of them have been called "biliary cirrhosis" at one time or another [2153]. They all have in common (1) enlargement of the liver; (2) enlargement of the portal tracts; (3) pericholangiolitic fibrosis; (4) delayed formation of septums and regenerative nodules, with relatively subdued regeneration owing to bile stasis. The three main causes are (1) intrahepatic cholestasis; (2) noninfected extrahepatic cholestasis; (3) infected extrahepatic cholestasis. These are present in several diseases discussed in different chapters (see Table 52). None is very frequently encountered in clinical practice, and some are medical oddities.

Table 52 Forms of Biliary Fibrosis and Cirrhosis and Their Relative Incidence

<i>Form of Disease</i>	<i>Cirrhosis</i>
Chronic biliary hepatitis due to extrahepatic obstruction.....	Common
Chronic biliary hepatitis due to extrahepatic atresia in children.....	Infrequent
Chronic biliary hepatitis due to intrahepatic atresia in children (acholangic).....	Very rare
Secondary biliary cirrhosis due to infected biliary hepatitis.....	Most common
Cholangiolitic cirrhosis.....	Infrequent
Cholangiolitic cirrhosis with xanthomas	Rare
Fibroxanthomatous cirrhosis of McMahon.....	Very rare

Reduction of the oxygen supply and the mechanical effects of passive congestion damage the hepatic cells. The main mechanical effect upon the hepatic-cell plates is increased pressure exerted by congested and dilated sinusoids. In many clinical examples, the contributions of anoxia and of mechanical pressure can not be determined. Moreover, the picture is often complicated by toxic or nutritional factors.

EXPERIMENTAL STUDIES

The separation of the functional and structural effects of hypoxia from the pressure effects of altered circulation has been accomplished in animal experiments.

Effect of Hypoxia on the Liver. The liver is extremely sensitive to oxygen want, as illustrated by the frequent occurrence of hepatic edema and

hydropic degeneration [55] (Fig. 154). Oxygen deficiency is a major factor in many hepatic injuries, even those of a chemical nature [833]. In animals, hypoxia causes alteration of hepatic proteins and other nitrogen fractions and impaired bilirubin excretion [172]. In many animals, hyperbilirubinemia results from anoxia caused by high altitudes or exhausting exercise [1170]. Breathing air low in oxygen increases Bromsulphalein retention in patients with preexisting liver disease [1705]. Impairment of hepatic function after anesthesia has been associated with anoxia [2366], possibly as a result of reduced hepatic blood flow [3013].

Effect of Congestion upon the Liver. The attempt has been made in animal experiments to produce passive congestion of the liver with resulting centrilobular atrophy, necrosis, and disappearance of cells [3439], similar to that seen in human

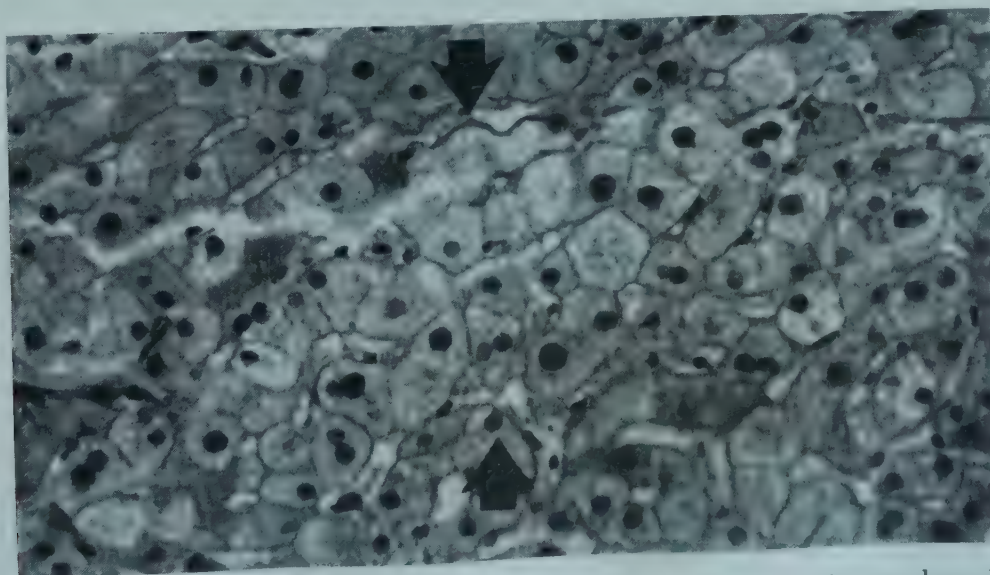


FIG. 154 Liver biopsy specimens from a patient 8 days after extensive esophageal hemorrhage, showing focal hydropic degeneration of the hepatic cells. H&E ($\times 285$).

passive congestion [2187, 3470]. The changes produced are caused either by increased venous pressure or by oxygen deficiency. The latter in turn is caused by slowing of sinusoidal blood flow or by reduced oxygen saturation because of diminished cardiac output or reduced pulmonary aeration [3681]. Probably several factors are responsible in each case [3040]. Some investigators feel that oxygen deficiency is more important initially [2655], although the pressure effect is more severe in acute congestion than in chronic congestion. The duration of the congestion is of greater importance than the degree. Improvement in heart failure reverses the changes [990, 3040].

Passive congestion, i.e., backward circulatory failure, results not only from cardiac failure with increased pressure in the inferior vena cava but also from constriction of the hepatic veins, either by inflammation (see Occlusion of the Hepatic Veins; Chiari's disease, later in this chapter) or by spasm of the veins [2625, 3463]. This contraction is often the result of toxic factors [2175, 3470]. Sometimes centrilobular congestion may result from spasm of the central vein alone. For instance, centrilobular blood stasis after experimental arteriolization of the portal vein seems to be caused by the resistance of the hepatic outlet veins to the increased flow of blood.

Hepatic congestion decreases inactivation of water- and salt-retaining hormones, leading to hypervolemia, which in turn increases the congestion, thus producing a vicious circle [961].

HUMAN HEPATIC INJURY FROM CONGESTION

Passive congestion of the liver in heart failure may result either from abnormalities of the heart itself or from lesions in other organs, especially the lungs, which affect cardiac function. The brunt of right heart failure is felt in the liver, since the distance from the right atrium to the hepatic veins is very short, and since the liver is a major blood depot. No reason has been found why congestion in the liver is increased out of proportion to that in other organs, or vice versa in some instances. In all cases, the liver is grossly enlarged and heavy. Its anterior edge is blunted, and the gallbladder bed is edematous.

Acute Passive Congestion of the Liver

In acute cardiac failure the liver enlarges, and the capsule, which is rich in nerves, becomes tense.

The organ is tender, especially in the gallbladder region, and sometimes causes spontaneous pain in the right upper quadrant. However, in the overall picture of acute congestive failure, the hepatic changes play an insignificant rôle, and jaundice is rare.

Grossly, the liver is large and heavy, and its anterior edge is blunted. On the cut surface of the liver, the lobular markings are accentuated, because the central zones are red and depressed, while the periphery is red-brown (Fig. 155, top).

The hepatic vein branches are dilated and the gallbladder bed is often edematous.

Histologically, in mild heart failure, severe hyperemia of the central zone is seen, and the central and sublobular veins are dilated. In more severe congestion, widely dilated sinusoids appear to compress the elongated hepatic-cell plates, predominantly in the central zone, and some hepatic cells in the central zone have disappeared. In very severe, sudden cardiac failure, for instance after rupture of a valve, a chorda tendina, or the interventricular septum, the hepatic cells rapidly disappear and the central zone is entirely replaced by red cells. However, the connective tissue framework is not disrupted. The extent of the destruction depends on the duration of the cardiac failure. Sometimes two-thirds of the lobule appears destroyed (Fig. 156, upper left) and only a small rim of intact hepatic cells surrounds the portal tract. When the lesion is far advanced, the lobular pattern is reversed, i.e., the parenchymal remnants in the periportal area are surrounded by confluent, congested central zones. Since these changes develop terminally, they are not necessarily associated with functional evidence of hepatic insufficiency.

Chronic Passive Congestion of the Liver

In subacute, or chronic, cardiac failure, i.e., of more than several days' duration, significant clinical and laboratory manifestations of hepatic-cell damage appear. Some of these disturbances are aggravated by toxic or nutritional factors, such as alcoholism or starvation [990, 3584].

Jaundice in Heart Failure. Deep jaundice is rare, but some hyperbilirubinemia is usually found, in most cases not exceeding subicteric levels [555, 1996, 3040, 3588]. The level of the serum bilirubin is not related to the severity of the cardiac failure nor to the extent of the histologic changes in the liver [3589]. Pulmonary infarcts [943] or

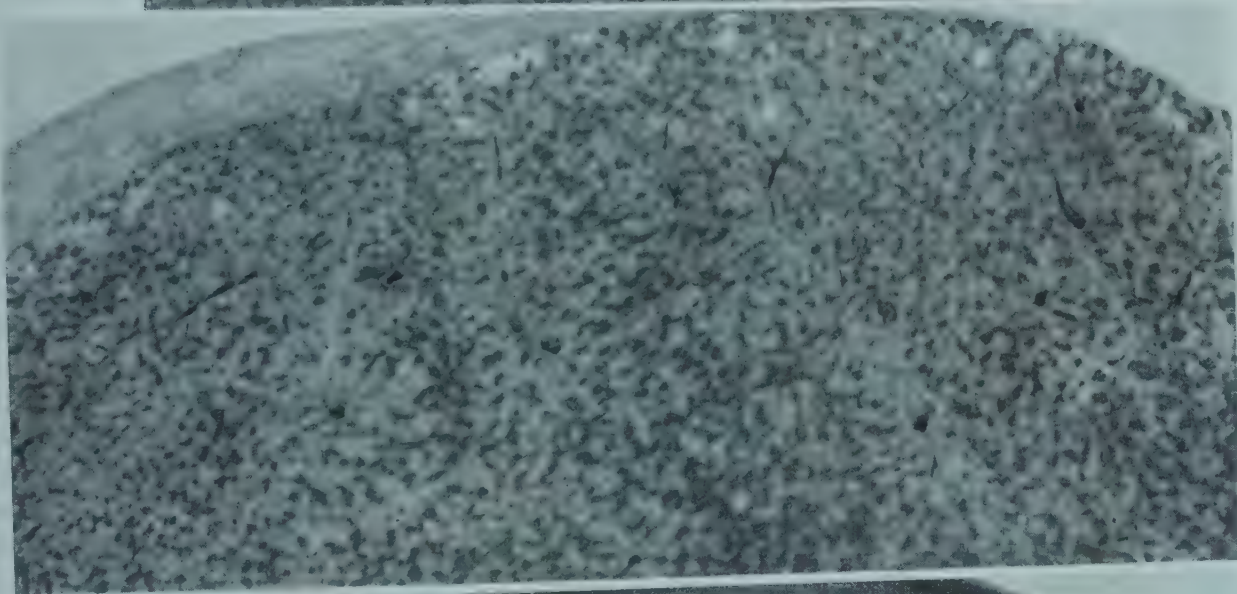
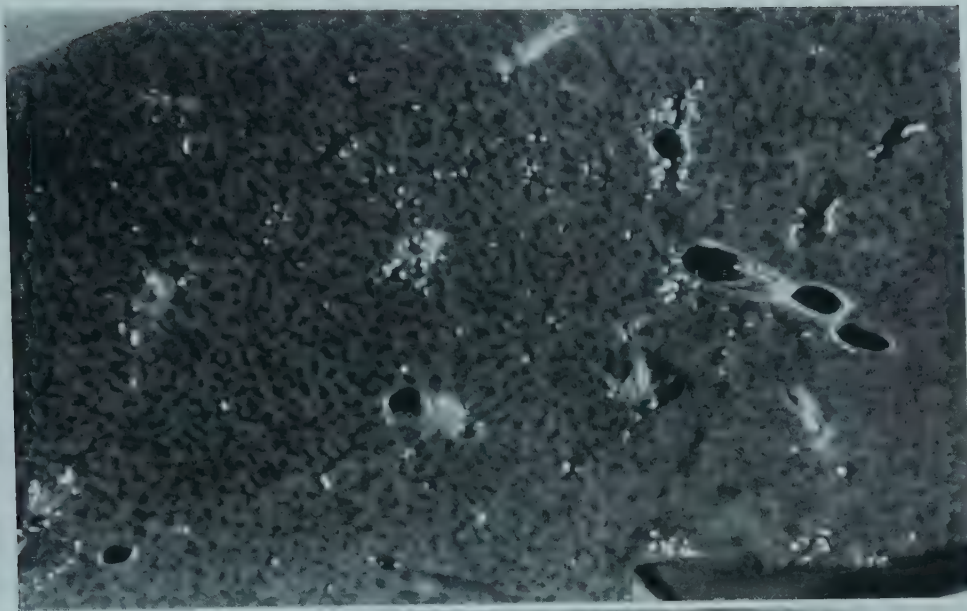


FIG. 155 *Top.* Acute passive congestion. The central fields are dark and depressed. *Center.* Subacute passive congestion. The depressed central fields are connected by hemorrhagic bridges (reversal of lobular pattern). *Bottom.* Small regenerative foci and some distortion of the lobular pattern are added to the changes just described for subacute passive congestion.

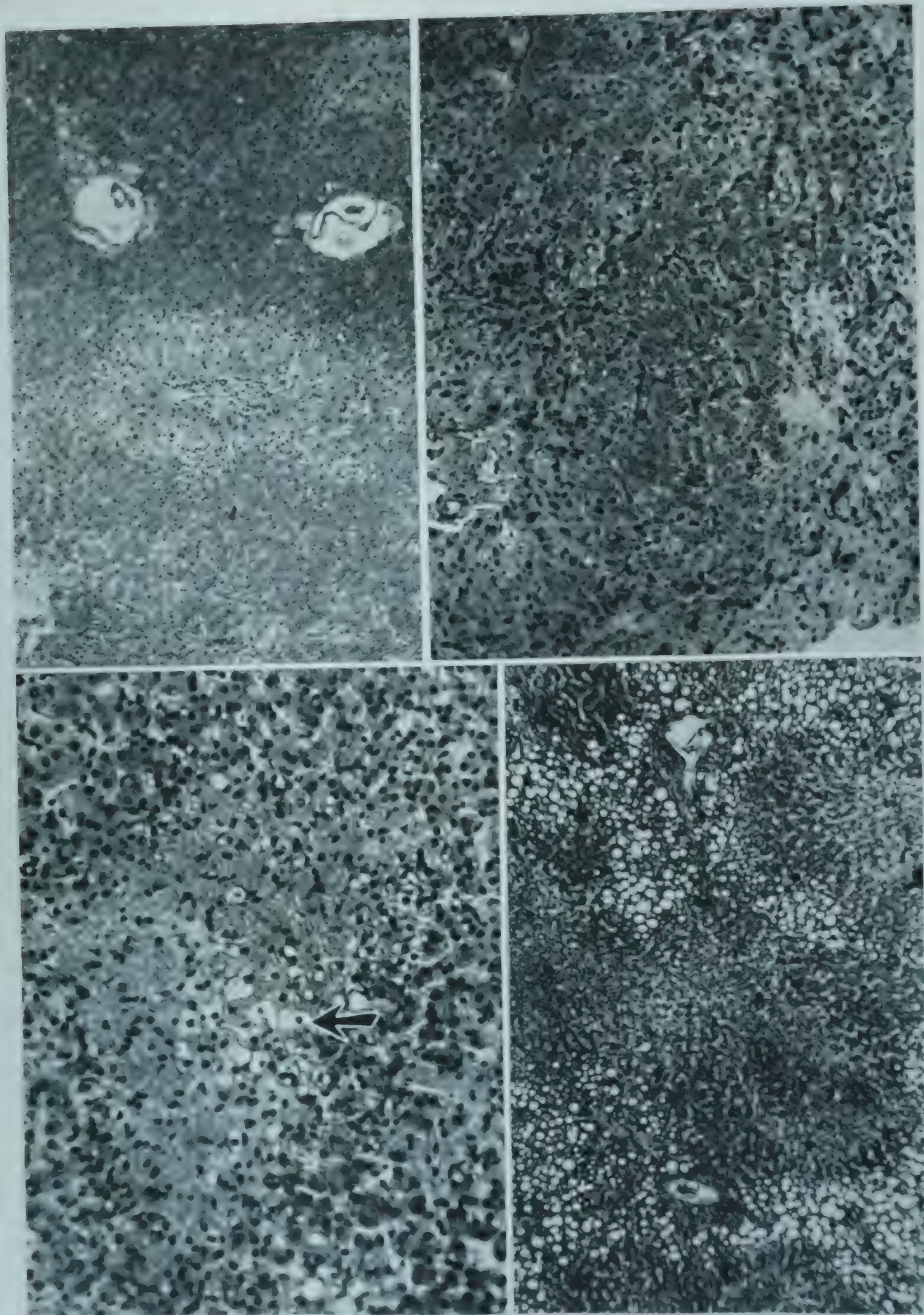


FIG. 156 *Upper left.* Severe acute passive congestion with hemorrhage almost entirely replacing the parenchyma, except for a small rim around the portal tracts. H&E ($\times 70$). *Upper right.* Biopsy specimen of subacute passive congestion, showing intrasinusoidal and extrasinusoidal accumulation of blood in the middle half of the lobule, with some persisting hepatic-cell plates. H&E ($\times 82$). *Lower left.* Biopsy specimen of acute passive congestion, with disappearance of hepatic cells from the lobular center and hydropic swelling of cells on the lobular periphery (arrow). H&E ($\times 150$). *Lower right.* Centrilobular hemorrhagic necrosis and fatty metamorphosis in the intermediate zone in subacute passive congestion. H&E ($\times 70$).

hemolysis [943, 1996, 3040] have been suggested as causes for jaundice, but the absence of infarcts at necropsy and the presence of bilirubinuria and increased prompt-reacting serum bilirubin suggest other factors. Hepatic-cell degeneration from compression of the cells and from toxic breakdown products in infarcts [943] has been considered. Compression of the bile canaliculi by the increased venous pressure has also been suggested [943] but is not substantiated by the laboratory findings [3040]. A varying combination of these factors probably is responsible for the jaundice, explaining the lack of correlation with functional or clinical observations.

Clinical and Laboratory Findings. The large and tender liver has a blunt edge. Some ascites is present but is not primarily the result of hepatic dysfunction. Increased Bromsulphalein retention is the most frequently seen abnormal laboratory finding [555, 961, 990, 2655, 3040, 3588, 3589]. This seems to be related to elevation of the venous pressure rather than to faulty oxygen saturation [961]. Urobilinogen is regularly increased in the urine; this fact has been considered useful in the evaluation of the degree of decompensation [555, 943] and in the diagnosis of myocardial infarction [960]. The other tests show more erratic results. Abnormal cephalin flocculation, thymol turbidity, serum alkaline phosphatase, total cholesterol, and cholesterol ester levels are found in less than half of the cases [961, 990, 2640]. Low total serum-protein and serum-albumin levels are found frequently [990, 3589] but are related to the accompanying poor nutrition. The changes usually respond to cardiac therapy but without sufficient regularity to permit prognostic conclusions, with the possible exception of Bromsulphalein retention and urobilinogen excretion. The correlation of the findings with morphologic changes is poor, again with the possible exception of Bromsulphalein retention [3040].

Biopsy Findings. The morphologic changes seen in biopsy specimens [3040, 3460, 3588, 3589] are compression of the hepatic cells in the center of the lobules by dilated sinusoids and sometimes edema fluid in the tissue spaces (Fig. 156, upper right). The hepatic cells vary in appearance; some show increased basophilia, explained by compression of the cells, while others contain acidophilic granules. The nuclei become pyknotic and fragmented, and the cells disappear. Persisting cell fragments are not found in the absence of toxic factors. In severe cases extensive necrosis, with

extravasation of red cells, is noted, and in such instances fibrin thrombi may be seen obstructing the sinusoids. The intensity of the changes decreases toward the portal area. The hepatic cells in the intermediate zone are sometimes hydropic (Fig. 156, lower-left). Fatty changes are common, especially in the central zone and on the border of the congested area. In some instances these changes are caused by malnutrition [3588]. The glycogen content is not reduced. The Kupffer cells show few changes except for phagocytosis of brown, usually iron-free, pigment, which originally was in the hepatic cells. If congestion persists longer, the connective tissue framework in the center of the lobule collapses and collagen membranes radiate from the central area into the lobular parenchyma.

Macroscopic Appearance. The liver varies in size, depending upon the duration of the congestion. In the subacute stage the liver is not so much enlarged as in the acute stage, although the edge is still blunt. The capsule is stretched, and on the cut surface in some areas the lobular architecture is exaggerated. In other areas the red and depressed lobular centers are connected with each other by bridges, resulting in a reversal of the lobular architecture. The apparent centers are the periportal areas, which often appear yellow, owing to fatty metamorphosis (Fig. 155, center). In chronic passive congestion, the liver is smaller, sometimes even smaller than normal, and the edge becomes sharp ("cyanotic atrophy"). The surface is somewhat irregular, and the capsule is often thickened and covered by white ridges or plates of organizing fibrin. The cut surface shows a polymorphous picture because of focal exaggeration or reversal of the architecture, fatty metamorphosis, and occasional small irregularly outlined regenerative nodules, usually yellow in color owing to fat deposition (the "nutmeg liver"). The hepatic veins are wide (Fig. 155, bottom). After subsidence of the congestion, the red hue disappears, but the architecture remains obscure, and the nature of the lesion is frequently difficult to recognize.

Histologic Changes. Several histologic stages have been described [331, 2187]. The lesions are more severe than the ones seen on biopsy specimens.

CENTRAL NECROSIS. Following the initial stage of central dilatation of the sinusoids with compressed basophilic hepatic-cell plates, a stage of central necrosis develops, with disappearance of hepatic

cells (Fig. 156, lower right). Fibrin thrombi are seen in the central zone. Red cells escape into the tissue spaces, and Kupffer cell mobilization occurs, associated with increased phagocytosis. Fatty metamorphosis develops in the zone adjacent to the necrotic area. If fatty metamorphosis is severe in the center of the lobule, congestion appears in the intermediate zone (intermediate congestion). The central necrotic areas in places are connected by bridges, as in the acute stage. This stage has caused extensive discussion as to the relative importance of congestion and toxic and nutritional factors [331, 2187, 3470], but little is gained in attempting to differentiate the effect of each factor in the individual case. Mercurial diuretics have often been accused of being toxic, but they do not seem to be of much importance in producing hepatic changes [1848].

CENTRAL COLLAPSE AND FIBROSIS. After disappearance of the hepatic cells, the reticulum framework in the lobular center collapses, and radiating collagenous membranes develop. The reticulum fibers appear thickened, and new fibers seem to form. Eventually the entire central zone is transformed into a fibrotic area, with a few mesenchymal cells (some of which are rich in iron-containing pigment), some proliferated ductules, and relatively few patent blood channels (Fig. 157, lower left). Differentiating these channels from hepatic vein branches is often difficult, because the veins exhibit fibrotic thickening, and their lumens are relatively narrow. Fibrosis of the vessel wall has been considered the result of rheumatic phlebitis [2798], and most instances of centrolobular fibrosis are found in rheumatic heart disease. However, the same fibrosis is seen in chronic hypertensive or arteriosclerotic heart disease. The fibrosis is most apparent after repeated episodes of heart failure. The areas of fibrosis are irregularly outlined, and eventually, after more than one-third of the lobule is involved, bridges develop between neighboring centrolobular zones, similar to the bridging of necrotic areas, reversing the lobular architecture and leaving the peripheral part of the lobule surrounded by a fibrotic shell [3040]. In this stage, inflammatory changes often develop in the portal tracts and the intact lobular periphery shows diffuse or nodular regeneration. Both processes further obscure the lobular architecture. In most instances of this stage, the picture is not uniform, because (1) the degree of congestion seems to vary in different parts of the liver; (2) focal acute exacerbations occur; (3) regenerative

attempts are not uniform; (4) the fibrosis varies; (5) toxic factors are often present.

The great variations throughout the liver partly explain the poor correlation between the structural changes and the clinical and laboratory findings on the one hand and the degree and duration of the cardiac failure on the other [1848]. In congenital heart disease with cyanosis, the liver is enlarged, and although the sinusoids are greatly dilated, the central veins are not, in contrast to the condition of other forms of congestive failure [1310].

Congestive Fibrosis of the Liver

Extensive hepatic fibrosis in prolonged cardiac failure is not sharply differentiated from the previous stage. It is defined as a condition in which excess connective tissue, partly resulting from collapse and condensation of reticulum and partly from new formation of fibers, is the predominant finding.

Functional Significance. Statistical studies indicate that repeated episodes of heart failure are an important factor, and that rheumatic heart disease is the most common cause, followed by hypertensive and arteriosclerotic heart diseases and cor pulmonale [331, 1133, 1702, 1832].

The liver in this stage is firm and not tender. It is often smaller than normal, but sometimes fibrosis compensates for parenchymal loss [1702]. Its size is not in keeping with the high venous pressure. The spleen is usually enlarged, as evidence of portal hypertension [2359]. In view of the importance of these congestive phenomena the term congestive fibrosis is preferable to cardiac fibrosis or cyanotic atrophy [1696]. Ascites is a common finding, but it is not explained by the portal hypertension alone. Esophageal varices are rare. Slight jaundice is almost always found in the cyanotic patients, while severe jaundice is rare. The laboratory findings are those of chronic congestion. Serum-albumin level is usually low, and Bromsulphalein retention is high. Urinary urobilinogen is increased, and bilirubin appears in the urine.

Structural Alterations. MACROSCOPIC APPEARANCE. Grossly, the liver is normal or slightly reduced in size. The surface and cut surface are irregular in color, as well as in architectural markings (Fig. 157, upper). The "nutmeg" designation is best applied to this stage, when the mottling is more severe and the lobular architecture can not be discerned. Fine strands of connective tissue are

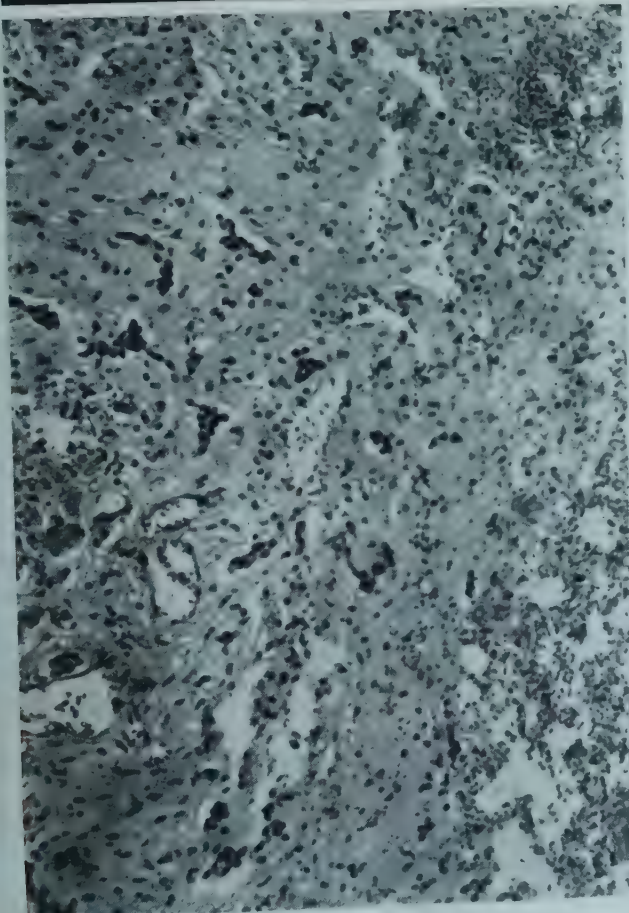


FIG. 157 *Upper.* Cardiac fibrosis distorting but not abolishing lobular architecture, which is in places reversed (arrow). *Lower left.* Centrilobular fibrosis in chronic passive congestion, with increased number of ductules extending almost to the central vein. H&E ($\times 120$). *Lower right.* Cardiac cirrhosis with loss of lobular architecture and dilatation of hepatic vein branches in chronic rheumatic heart disease with multivalvular involvement, including organic tricuspid incompetence.

verse the liver, and red, depressed, small foci of collapse can be seen where lobules or parts of them have disappeared. Garland-shaped and irregular regenerating areas differ in color from the surrounding tissue. The liver is hard, and resistance to cutting is increased. The capsule is usually thickened, and the changes in the subcapsular zone are the most severe, supposedly because of the increased pressure exerted by the stretched capsule [2359]. The gallbladder bed appears scarred.

HISTOLOGIC CHANGES. The changes of the earlier stages are accentuated, and the appearance is polymorphous [849, 1702, 1833, 1848]. The lobular architecture, although obscured, is generally intact. An irregular fibrotic tissue extends from the center, replaces part of the lobule (Fig. 157, lower left), and connects central portions of neighboring lobules. Portal fibrosis is considerably more extensive than in the previous stage [1702], but whether it results from irritation by cellular breakdown products or from an independent insult to a damaged liver is unknown. Whether the fibrosis results from mere condensation [1702, 1848] or from active new formation of fibers and even capillaries [2359] is also unsettled. Collapse of the framework after disappearance of the hepatic cells is probably the initial factor, followed by the development of collagen membranes. Relatively little new formation of fibers by fibroblasts occurs. Most of the vessels permitting blood to pass from the portal to the central areas run through preformed capillaries. The remaining liver parenchyma shows evidence of regeneration, producing fairly severe nodularity, without abolishing the lobular architecture. Portohepatic venous anastomoses are rare, and the newly formed nodules do not compress hepatic veins. Therefore the term "cirrhosis," widely applied in this stage [1702, 1832, 1848, 3040], is best avoided [331, 2359].

Congestive, or Cardiac, Cirrhosis

True congestive, or cardiac, cirrhosis is a rare lesion which, with very few exceptions, develops only in constrictive pericarditis or in long-standing tricuspid incompetence [912, 2359]. Under these circumstances, the hepatic congestion becomes severe enough so that the central fibrosis in many areas extends to the portal tracts. This leads to portohepatic venous anastomoses and to destruction of the lobular architecture. The lobule is now dissected, and regenerative nodules form, leading to compression of the hepatic vein and portal hy-

pertension. Under these circumstances, progressive cirrhosis develops. The laboratory findings are those of chronic hepatic failure. Grossly the liver is smaller than normal and finely granular (Fig. 157, lower right). The garland-shaped arrangement of the hepatic parenchyma in parts of the liver and, histologically, the central predominance of the fibrosis in less cirrhotic areas serve to differentiate this lesion from diffuse septal cirrhosis. Diffuse septal cirrhosis resulting from malnutrition or alcoholism associated with cardiac failure is fairly common [3589].

Perihepatitis

In a group of diseases which have in common chronic effusion in one or more of the serous cavities, a diffuse thickening of the hepatic capsule is noted. This is caused by organization and hyalinization of fibrin, resulting in a thick white coating (the Zuckerguss liver) [2804]. Several factors are responsible for the change:

1. Exudative pericarditis, or constrictive mediastinopericarditis, interferes with the venous return from the liver by compressing the inferior vena cava.
2. Progression of the inflammation of mediastinopericarditis through the diaphragm to involve the diaphragmatic surface of the liver gradually produces a hyalinizing exudate, especially around the main hepatic vein branches.
3. Exudation from lymphatic vessels in Glisson's capsule in various forms of hepatic congestion associated with ascites results in precipitation of a protein-rich exudate over and below the capsule of the liver.
4. Extension of a generalized or localized chronic nonspecific, tuberculous, syphilitic, or neoplastic peritonitis to the surface of the liver produces a hyaline coating.

The functional significance of the perihepatitis depends upon the underlying disease. Quite frequently it is an incidental finding at autopsy, together with perisplenitis. In other instances it may be significant. For example, in pericarditis, the severe congestion of the liver may be aggravated by an associated perihepatitis. The thickened capsule exerts pressure, which interferes with circulation, especially on the periphery of the lobule.

In the perihepatitis associated with peritonitis or lymphatic obstruction, the fibrosing process along Glisson's capsule may extend into the liver for a distance of a few millimeters, resulting in subcapsular cirrhosis.

Pick's pseudocirrhosis is the hepatic component of polyserositis, or Concato's disease. It chiefly occurs in younger people and is characterized by recurrent ascites, a large firm liver, slight degree of jaundice or none at all, and leg edema. The hepatic involvement is mainly of anatomic rather than of functional or of clinical significance.

Curschmann's disease is chronic hyperplastic perihepatitis without pericardial involvement. In its pure form, only pericapsular, and no subcapsular, fibrosis is present, but the difference is just an expression of the associated congestion [2804].

Occlusion of the Hepatic Veins; Chiari's Disease

Interference with hepatic circulation by hepatic vein occlusion rarely occurs in man. It has been called Chiari's disease or the Budd-Chiari syndrome. Not many more than 100 instances have been reported in the literature [1500, 1722, 2513, 2606, 3327].

Structural Changes. The location of the obstruction may be in the intrahepatic portion of the inferior vena cava or, most frequently, in the large hepatic veins near their entrance into the vena cava, or in the smaller tributaries. In the last-named location, the process is usually only local and therefore of minor clinical interest.

Etiology. The lesion results from (1) abnormal processes in the vicinity encroaching upon the hepatic veins [2513]; (2) primary vascular disease; (3) spontaneous thrombosis; (4) congenital defect or malformations of the veins seldom confirmed at autopsy. Progression of a tumor or abscess, with or without thrombosis [2804], and thrombosis associated with carcinoma metastases are usually terminal events of little clinical interest. Carcinomas in the liver rarely invade the hepatic vein. Syphilitic phlebitis or periphlebitic gummas belong to the group of primary diseases leading to hepatic vein occlusion and were originally assumed by Chiari to be the cause of this disease [2804]. Other types of phlebitis, especially rheumatic phlebitis, are more commonly responsible [670]. In tropical liver injury, scarring of hepatic vein branches, or venoocclusive disease, occurs, probably from toxic damage rather than from nutritional changes [379] [see *Infantile Sclerosis (Venocclusive Disease of the Liver)*, under *Tropical Malnutrition*, Chap. 51]. A similar lesion is found in experimental senecio poisoning [3005]. The most common cause is hepatic vein thrombo-

sis in an apparently unchanged vein. This occurs as a result of (1) polycythemia vera [1048]; (2) compression of the veins in cirrhosis by hyperplastic nodules [2804]; (3) retrograde embolization, mentioned in the earlier literature and proved by the retrograde development of hepatic abscesses [564]; (4) obliterative endophlebitis, the largest group, the etiology of which is not established. Thrombosis may be part of a generalized recurrent idiopathic thrombophlebitis [1533, 2606], or it may involve only the hepatic vein, supposedly as a result of eddy formation in this location [3327].

In acute cases of a few days' duration, obstruction of the veins occurs, while in chronic cases they are stenotic. Histologically, severe hepatic congestion is noted, progressing to cardiac fibrosis and cirrhosis. In protracted instances severe lymphedema of the liver is present [2280], with patchy infarction throughout the organ [1533].

Clinical and Laboratory Findings. No age or sex preponderance is found in this condition. The duration varies from a few days to several years.

The clinical manifestations are often overshadowed by those of the underlying disease. The symptoms and signs pertaining to hepatic vein thrombosis are hepatic enlargement with tenderness in the acute phase, followed by shock and coma. Massive ascites, with minimal to moderate splenic enlargement and dependent edema, is usually found. Jaundice, if present, is mild. Venous collaterals in the abdominal wall are related to the duration of the obstruction and to the extent of the involvement of the inferior vena cava, and they extend more toward the upper abdomen and lower thorax than in uncomplicated cirrhosis. Pain over the liver, especially early, results from stretching of the capsule. Vomiting is common, as is hematemesis from ruptured esophageal varices. The normal venous pressure and circulation time differentiate this lesion from congestive failure; the rapid reaccumulation of ascites, from cirrhosis; the flow in the abdominal collaterals, which fill downward, from occlusion of the inferior vena cava; and enlargement of the liver, from portal vein thrombosis.

Laboratory findings are those of hepatic failure. The available data are erratic; the drop in cholesterol esters has been emphasized. Many patients die in hepatic coma [2804].

HEPATIC INJURY FROM DISTURBED CIRCULATION AND ANOXIA

The function and structure of the liver are altered in various conditions other than primary hepatic ones because of disturbed intrahepatic circulation as well as anoxia of the hepatic tissue. Anoxia produces focal hydropic swelling (see Increased Water Content, under Anoxic Necrosis, Chap. 22); when anoxia is severe and prolonged, it produces central and, more frequently, paracentral necrosis. In the individual instance, it is difficult to decide whether disturbed circulation or anoxia is more significant. In addition, endogenous toxic factors may play a role, the nature of which is not clearly understood.

Classification

- Hepatic necrosis from shock
- Hepatic congestion in thyrotoxicosis
- Hepatic necrosis in eclampsia
- Hepatic degeneration from hemolysis
 - Hemolytic anemia
 - Hemolytic disease of the newborn
 - Sickle-cell anemia

HEPATIC NECROSIS FROM SHOCK

Although the vascular dynamics in shock are unsettled, it is known that in all types of shock the circulation through the liver is impaired.

Etiologic factors of shock include secondary traumatic shock, postsurgical shock, toxic shock, hemorrhagic shock, and myocardial infarction [588].

Mechanisms Contributing to Hepatic Necrosis in Shock. The following hepatic mechanisms that contribute to necrosis in shock have been described:

1. A throttle mechanism in the hepatic vein, stimulated by shock, reduces the outflow of blood

from the liver and produces portal stasis. In the dog, this is accomplished by muscular sphincters in the hepatic vein branches [1097, 3323] (Fig. 53, lower left). In man circumscribed sphincters are absent, but the narrowed openings of the piercing veins into the larger, thicker-walled hepatic vein radicles act as sluices, particularly if the larger vein contracts [2625] (see Hepatic Vein, and Regulation of Hepatic Blood Flow, Chap. 18 (Fig. 53, upper left and upper right).

2. Increased permeability of the sinusoids, with resulting edema and increased lymphatic flow, is an important feature of shock produced by histamine or substances with a histaminelike reaction, such as anaphylaxis or peptone shock [945, 2248].

3. The anoxic liver tissue produces a humoral vasodepressor material (VDM) which is inactivated by normal liver tissue [3056].

Viviperfusion of the liver prevents irreversibility of hemorrhagic shock [1065], while preexisting hepatic damage predisposes to shock.

The following extrahepatic factors are responsible for the hepatic changes in shock:

1. Reduction of circulating blood volume
2. Anoxia, especially in hemorrhagic shock
3. Toxic effects of substances responsible for shock

Variable participation of these factors makes for poor correlation between the degree and duration of shock and the anatomic changes found in the liver [459, 914, 2191].

Functional Hepatic Changes in Shock. CIRCULATORY EFFECTS. The hepatic and portal venous blood flow and oxygen saturation [2158] are sharply reduced in experimental hemorrhagic shock [1066, 3597]. After exposure of the abdominal organs, with moderate loss of blood, the blood flow through the liver was reduced in cats and

dogs [934, 1447], with simultaneous reduction of bile production [937]. The disturbance is either part of a generalized circulatory effect with a reduced blood supply to the hepatic artery and the portal vein, or the result of circulatory factors operating within the liver.

METABOLIC EFFECTS. In the liver, metabolic derangements include (1) reduced oxygen consumption, observed in liver slices [2855], although increased consumption has also been reported [646]; (2) a decrease in high-energy phosphate compounds [1951]; (3) enzyme reduction and disappearance of glycogen [1064]; (4) reduction of hepatic sulfhydryl [201]. In the serum they include (1) increased amino acid nitrogen, lactic and pyruvic acids, and ammonia [934, 937]; (2) impaired galactose tolerance [1597]; (3) impaired regeneration of serum albumin, prothrombin, and fibrinogen [1064]. Bromsulphalein clearance is usually reduced, although not necessarily to a degree commensurate with the degree of shock [3689]. The serum-bilirubin level is elevated, especially in postoperative shock. Many other factors are involved in the postoperative period, and abnormal results in various hepatic tests are fairly frequent in this period in the absence of shock [1142].

CLINICAL CONSEQUENCES. In clinical shock, hepatic failure is of little obvious influence, but the ensuing widespread metabolic derangements suggest that the degree of hepatic involvement sometimes becomes the determining factor in the final outcome.

Structural Changes. **EXPERIMENTAL SHOCK.** The anatomic findings depend upon the type of shock. In histamine or anaphylactic shock, capillary damage usually prevails over congestive and anoxic phenomena. Hepatic-cell damage is minimal, but focal necrosis is noted [85, 1402], supposedly as a result of endothelial rather than of parenchymal changes [432]. In the dog, spasm of the hepatic veins produces stasis in the central sinusoids, formation of hyaline thrombi [3522], and parenchymal damage. Repeated histamine shock results in central hepatic fibrosis, which can be considered a reflection of both central vein contraction and increased capillary permeability [945]. In hemorrhagic or neurogenic shock, focal necrosis develops, characterized by nuclear alterations and disintegration of cells. When shock lasts longer than 24 hours, central necrosis develops [459, 914], caused by both central congestion and confluence of areas of focal necrosis.

HUMAN SHOCK. The structural changes in clinical shock reflect the effects of forward circulatory failure in the liver, from reduced portal and arterial blood flow, and backward failure, from narrowing of the hepatic vein [3470]. This has been described in detail in malaria [2174]. Focal necrosis and blood extravasates are frequent. In addition, central necrosis develops, associated with congestion and hemorrhage and later with collapse. Shock is one of the most common causes of central necrosis in many diseases [914, 3470] and explains the occurrence of central necrosis in acute toxic nephrosis and uremia [725]. Premortal shock is also the reason for the presence in autopsy specimens of central necrosis not found in biopsy specimens which were taken before death [2625, 2801, 3541].

HEPATIC CONGESTION IN THYROTOXICOSIS

Relation between the Liver and the Normal Thyroid Gland

Influence of the Thyroid on the Liver. **EFFECT ON REGENERATION.** In thyroid-fed animals, the liver is larger than normal [2128] and the hepatic cells are hyperplastic and basophilic [1841]. Single injections of thyroxin also enlarge the liver and increase the hepatic proteins [3211] and nucleic acids [486]. The thyroid gland has been said to be a regulator of hepatic regeneration, which is impaired in thyroidectomized animals [823, 943], although thiouracil increases the rate of regeneration [1041].

METABOLIC EFFECTS. As part of its general metabolic effect [157], thyroid hormone influences the cytochrome mechanism [823], ATP [3419], and oxidative phosphorylation [2185] in the liver. Thyroidectomy prevents the development of the fatty liver from lipogenic diets [3050]. Phospholipid turnover in the liver is increased by thyroxin and decreased by thiouracil [1035]. Hepatic glycogen is reduced by thyroid administration, an effect which has been utilized in assaying the potency of thyroid preparations [943]. The decrease is partly the result of reduction of lactic acid dehydrogenase activity, a specific effect of thyroxin, since other hepatic enzymes are increased rather than decreased [3422]. Vitamin B complex preparations, primarily vitamin B₁₂ [949], counteract this effect, along with other manifestations of experimental hyperthyroidism [833].

EFFECT ON HEPATIC STRUCTURE. Many histologic changes have been produced in animals, depending upon dosages and other experimental circumstances [1331, 1841, 3211]. The changes vary from necrosis of individual hepatic cells to edema, atrophy, and focal and central necrosis. Fat deposition is localized to the central zone, in which fatty cysts develop [3001]. These alterations are severely aggravated if excessive thyroid feeding is combined with chloroform intoxication [2129], various infections [1331, 2980], or anoxia [2129]. Such factors also produced the lesion if combined with administration of amounts of thyroid otherwise innocuous.

EFFECT OF HYPERTHYROIDISM. Experimental information about the effect of hyperthyroidism upon the liver is confusing. Susceptibility to toxic or infectious injuries seems to be increased, possibly because the raised metabolism produces a relative deficiency of protective factors. The hepatic circulation seems to be disturbed, but the evidence is based on morphologic observations which are complicated by cardiac failure from thyrotoxicosis, rather than on experimental observations. Measurements of hepatic circulation in patients with hyperthyroidism indicate that hepatic blood flow is only slightly elevated, although the cardiac output is greatly increased and the splanchnic oxygen consumption is increased out of proportion to the raised metabolic rate. In hyperthyroid animals increased Bromsulphalein clearance [837] and reduced galactose tolerance are found.

The evidence for a direct toxic effect of thyroid hormone upon the liver is the decrease in lactic acid dehydrogenase and, subsequently, glycogen in the liver [3422]. The excretion of cholesterol in the bile is under the influence of the thyroid hormone, as evidenced by the fact that the excre-

tion is increased in hyperthyroid rats and reduced in hypothyroid ones [2823]. This correspondingly affects the serum-cholesterol level. Hepatic injury in clinical hyperthyroidism thus appears to be only to a minor degree the result of thyroid hormone itself; it is rather the effect of the synergistic action of various factors, of which the most important are congestion, infections, and toxins to which the liver is more susceptible (Fig. 158). The role of congestion is the reason for including changes in the liver from hyperthyroidism in this section. Structural alterations tend to be localized in the central zones because of a relative oxygen deficiency at this site [2398]. The effect of hypothyroidism on the liver concerns the beneficial effect of antithyroid drugs in cirrhosis [1320] and the alteration of fat distribution by reduced requirements [3001].

Influence of the Liver on the Thyroid Gland. The level of serum-precipitable iodine is elevated in early stages of hepatitis but is low or normal in cirrhosis and normal in obstructive jaundice, possibly because of a specific metabolic function of the liver [1890]. The liver is one of the most important sites of accumulation of injected labeled thyroxin [1294]. It is converted to an unknown iodine-containing compound and excreted in the bile [401, 3302]. The excretion is increased in hyperthyroid animals and reduced in hypothyroid animals [1803]; whether this applies to man is questionable [1646].

Manifestations of Hepatic Injury in Thyrotoxicosis

Since cardiac failure frequently complicates severe thyrotoxicosis, it is difficult to evaluate whether hepatomegaly is caused by excess thyroid hormone, although it occurs in some instances without cardiac involvement [271, 3473]. The

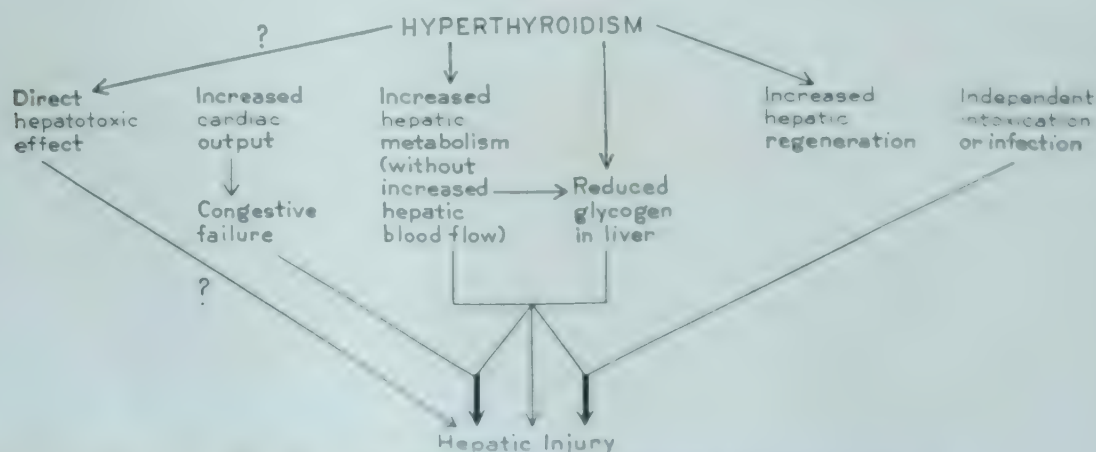


FIG. 158 Pathogenesis of hepatic injury in hyperthyroidism.

incidence of jaundice is low [1996, 2128, 3015]. When it occurs, it is most probably caused by cardiac failure or other conditions to which the liver is more susceptible.

Laboratory Findings. The several factors usually responsible for hepatic injury often produce abnormal results of hepatic tests. They parallel the clinical and laboratory findings of hyperthyroidism, and normal hepatic function is restored after thyroidectomy [1996, 2150]. Results of the oral galactose-tolerance test are best correlated with the clinical findings [54, 2128]. Under these circumstances, abnormal tolerance is caused not only by hepatic injury but also by increased intestinal absorption not related to hepatic insufficiency [53]. Hepatic injury alone is indicated by the intravenous galactose-tolerance test. Glucose tolerance is also impaired [2936]. The response of the carbohydrate metabolism, especially galactose tolerance, has been used as a supplement in the management of thyrotoxicosis. The abnormal results do not imply a generalized hepatic injury but rather a specific effect on glycogenesis characteristic of this disease. Abnormal results of other hepatic tests, such as impaired hippuric acid synthesis [1343, 1652], hypoprothrombinemia [2067], and Bromsulphalein retention, are less frequently found. The reduction of serum cholesterol in hyperthyroidism is independent of any hepatic injury and is apparently related to increased cholesterol excretion in the bile [2823].

Structural Alterations. Structural changes found in hyperthyroidism in man at autopsy include fatty metamorphosis, edema, interstitial infiltration, diffuse and massive hepatic necrosis, and cirrhosis [467, 1331, 3015]. The changes are correlated with the severity and duration of the clinical manifestations but, with the exception of one rare form of cirrhosis, they are not specific and are related to accompanying factors which are often present in hyperthyroidism or possibly to increased susceptibility of the liver to injury. Hepatic edema, or serous hepatitis [2798], is partly caused by stasis. The instances in which necrosis was reported were possibly caused by either associated congestion or an independent intoxication or infection.

TYPES OF CIRRHOSIS. Three types of cirrhosis are observed in thyrotoxicosis: (1) cardiac fibrosis or cirrhosis with central fibrosis and capsular thickening, caused by cardiac failure; (2) nonspecific septal cirrhosis, sometimes with fatty infiltration, presumably nutritional or toxic in origin; (3) a

specific type of cirrhosis characterized by severe congestion, thickening of the capsule with subcapsular exaggeration of the changes, and portal rather than central fibrosis [1331, 2798]. It is considered to be caused by circulatory failure with increased cardiac output, rather than by chronic hepatic venous congestion. Because of the increased velocity of the arterial blood flow, hydrodynamic difficulties have been assumed to develop where portal blood and hepatic artery blood mix, especially on the periphery of the portal tracts, causing fibrosis in this area. The capsule is thicker and the subcapsular changes more prominent than in cardiac fibrosis. This fairly specific type of cirrhosis is not the one most frequently found in hyperthyroidism, nor is cirrhosis an important complication.

BIOPSY FINDINGS. Liver biopsy findings in hyperthyroidism are disappointing [2367, 2601]. The only significant alterations encountered are non-specific reactive hepatitis and some vacuolization of the hepatic-cell nuclei related to disturbance of the carbohydrate metabolism (see Nucleus, Chap. 3).

Therapeutic Considerations. Since thyrotoxicosis frequently is associated with hepatic alterations as a result of increased susceptibility of the liver to other toxic injuries, specific therapy for existing or potential liver injury has been recommended [2128, 2746, 2936]. This appears justified if the results of hepatic tests are abnormal.

Thyroid Crisis. Thyroid crisis is often associated with hepatic injury [2128], which has even been considered to be the cause of the crisis [362, 3015]. At autopsy central necrosis is frequently found, but this is probably a result rather than the cause of the crisis. No causative relation between thyroid crisis and hepatic injury can be assumed [2097].

HEPATIC NECROSIS IN ECLAMPSIA

Pathogenesis. In the group of toxemias of pregnancies which are designated as eclampsia, the liver is involved in at least half the cases [779]. The hepatic lesion was originally thought to be the result of a toxin, but circulatory factors and alterations of blood coagulation now appear to be more important. Large veins may occasionally be obstructed by thrombi, resulting in infarcts, but the characteristic alteration is the presence of fibrin thrombi in the sinusoids, especially near the portal tracts. These thrombi obstruct the sinus-

oidal blood flow and produce necrosis of hepatic cells, with resulting hemorrhage and occasional rupture of the framework. The peripheral zone of the lobule in the vicinity of the portal tract becomes anoxic, since the central zone receives blood from other sinusoids, and peripheral necrosis results. The etiology of these fibrin thrombi, also found in the kidney and the pituitary and adrenal glands, is thought to be release of thromboplastic material from the placenta, which is often altered in eclampsia [2945]. Increased formation of fibrin thrombi, with subsequent consumption of circulating fibrinogen, in addition to an increase in fibrinolysin titer, explains the frequent fibrinopenia with bleeding tendencies. A combination of peripheral necrosis and hemorrhage in the liver thus is pathognomonic for eclampsia.

Various unsuccessful attempts have been made to simulate experimentally the eclamptic changes of the liver by interference with the blood supply, by raising the intraabdominal pressure, by injection of tissue extracts containing fibrinogen, and, recently, by injecting olive oil [3301]. The best results have been obtained by progesterone injection into pregnant rats [3284].

Clinical Manifestations of Hepatic Changes. No correlation is found between the severity of the clinical manifestations and the anatomic findings in the liver. In typical eclampsia with convulsions and changes in other organs such as the kidney, the liver may be uninvolved, or it may show only terminal changes probably of little functional significance. On the other hand in eclampsia with few or no convulsions, the hepatic changes may be most severe, possibly because the lesion progresses farther in the absence of warnings [791]. Jaundice in eclampsia is rare and suggests a grave prognosis. The nature of the hepatic changes in other toxemias of pregnancy, such as hyperemesis gravidarum, is not so well established. Even in those rare instances in which jaundice appears, the morphologic changes in the liver may be insignificant [2448].

Laboratory Findings. Earlier studies indicated impairment of hepatic function in toxemias of pregnancy, but the practical clinical significance of this conclusion is questionable. In eclampsia, the serum proteins are altered. This alteration is responsible for abnormal results of the flocculation and turbidity tests [748], and abnormal cephalin flocculation during pregnancy has been claimed to herald approaching toxemia. Slightly increased Bromsulphalein retention may be found. The

serum-uric acid level is elevated out of proportion to the nonprotein nitrogen [1236, 3521]. The reason for this is not known.

Structural Alterations. **MACROSCOPIC APPEARANCE.** The liver is usually large, and the capsule is tense. Throughout the otherwise pale liver hemorrhagic red or gray areas are seen. These areas vary in size: some are barely visible, others are several centimeters in diameter, predominantly in the subcapsular portion of the right lobe (Fig. 159, upper). These are irregularly outlined and appear sunken on the cut surface. Occasionally a subcapsular hemorrhage ruptures, causing hemoperitoneum [2882]. Sometimes gross manifestations are entirely absent, despite severe histologic damages.

HISTOLOGIC CHANGES. Fibrin thrombi are seen throughout the liver, even in uninvolved areas (Fig. 159, lower right). They are often found in the center of small areas of necrosis involving only a few cells. This is wrongly called "fibrinoid necrosis"; it is more likely acidophilic necrosis, owing to loss of PNA [294, 3521]. In larger necrotic foci, the hepatic cells subsequently disappear and blood pools develop. Fragments of necrotic tissue seem to float in these pools, in which the framework also ruptures. The hemorrhagic areas are mainly portal in distribution if they are small, but as they become larger they involve whole lobules or many lobules. Fibrin precipitates are seen in these larger pools. Inflammatory reaction is characteristically absent even from larger lesions, indicating that they develop only shortly before death. In some instances, the hemorrhages are in the background and necrosis predominates (Fig. 159, lower left). Sometimes centrilobular necrosis is seen; it results from a generalized disturbance of sinusoidal blood flow, rather than from a local obstruction. This is accounted for by the shock so frequently present in patients with eclampsia. In liver biopsy specimens no significant changes were observed in pre-eclampsia, while in fully developed stages, necrosis, hemorrhages, and fibrin precipitates have been found [1588, 2448]. Healing has also been observed.

OTHER HEPATIC CHANGES IN PREGNANCY. Liver damage in eclampsia must be differentiated from other types of hepatic injury occurring in pregnancy and associated with jaundice, such as viral hepatitis, with typical biopsy findings [2448] toxic hepatitis [3033]; an apparently rare fatal acute fatty metamorphosis associated with focal necrosis and explained as a metabolic disturbance.

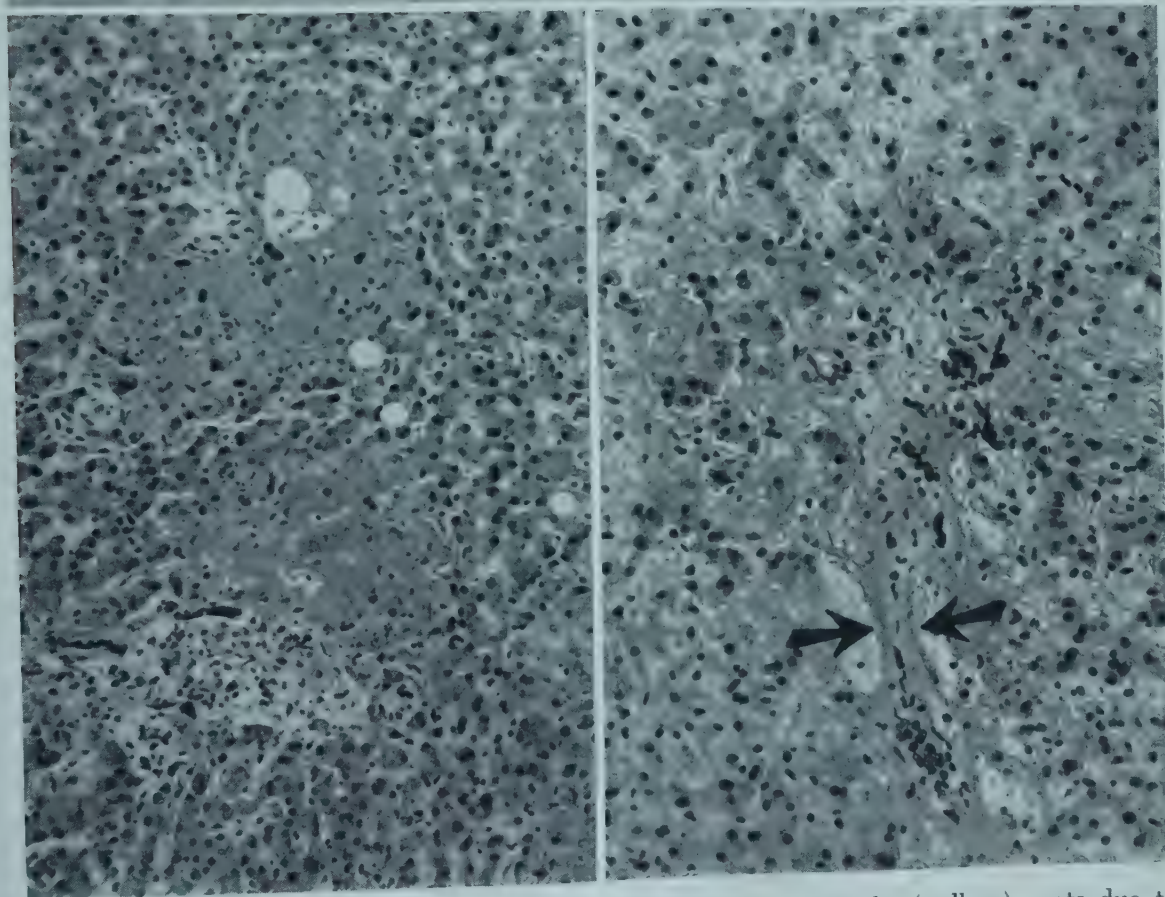


FIG. 159 The liver in eclampsia. *Upper.* Note irregularly shaped light (yellow) spots due to necrosis. *Lower left.* Periportal foci of hemorrhagic necrosis and disappearance of hepatic cells. H&E ($\times 85$). *Lower right.* Fibrin films in capillaries near portal tract in the necrotic zone (arrows). H&E ($\times 150$).

[2468]; and hemolytic jaundice or extrahepatic biliary obstruction by calculi.

HEPATOCELLULAR DEGENERATION FROM HEMOLYSIS

Increased hemolysis, especially if associated with jaundice, is reflected by bile pigment deposition in Kupffer cells and hepatic cells and by the presence of bile casts. When hemolysis is chronic or repeated, hemosiderosis is noted (see Siderosis, or Hemosiderosis, Chap. 53). These changes do not impair hepatic function. The only abnormal results of hepatic tests are elevated level of indirect-reacting serum bilirubin and increased urinary urobilinogen, caused by increased hemoglobin breakdown (see Bilirubin and Urobilinogen in Urine, Feces, and Bile, Chap. 36). Abnormal results of other tests are not necessarily associated with structural changes.

Anemia as such produces centrilobular fatty metamorphosis [2863] and, in more severe cases, centrilobular necrosis as a result of local hypoxia. This is aggravated by the hemolysis, especially when jaundice is present, because (1) biliary drainage is disturbed by bile plugs (see Intrahepatic Biliary Obstruction, Chap. 21) or by extrahepatic biliary obstruction resulting from pigment stones [3533]; (2) the liver may be injured by toxic blood-breakdown products [2625]; (3) blood flow is disturbed by either enlarged Kupffer cells or by intravascular conglutination of red cells. If an antigen-antibody reaction is responsible for the hemolysis, it may also produce hepatic-cell damage [2611].

Such alterations can occur in all types of hemolytic anemias, particularly the chronic ones. They are more severe in the hemolytic diseases of the newborn and in sickle cell anemia, both of which produce severe hepatic lesions.

Congenital and Acquired Hemolytic Anemia

Liver disease has been considered one of the possible causes of hemolytic anemia with jaundice [943, 3502], and viral hepatitis has been assumed to precede some instances of hemolytic jaundice [1677]. A hemolytic component in liver disease is probably present, as indicated by reduction of the life span of the red blood cells. However, the liver is not necessarily involved in most instances of hemolytic anemia, except for the presence of bile casts and bile imbibition and iron deposition in Kupffer cells. Increased urinary urobilinogen

excretion may be the only abnormality in the hepatic tests. Nevertheless structural changes are frequently found. They are not specific and include fatty metamorphosis, focal necrosis, and fibrosis [1584, 2237, 3165]. Some of the changes may be caused by hypoxia, which results from the anemia. Elevated levels of prompt-reacting bilirubin and bilirubinuria are usually thought to result from complicating hepatic damage.

Recent biopsy studies in patients with hemolytic anemia revealed some instances in which cirrhosis or necrosis was present, in addition to hepatic conditions such as sarcoidosis, Gaucher's disease, and lymphomas, i.e., symptomatic hemolytic anemia [1585]. These patients had enlarged livers, abnormal cephalin flocculation and thymol turbidity, increased alkaline phosphatase, and elevated level of prompt-reacting bilirubin. In this group with liver damage, splenectomy yielded poor results.

Hemolytic Disease of the Newborn

The effects of hemolytic anemia on the liver are exaggerated in the group of diseases described as erythroblastosis fetalis because of the young age of the patient and the immaturity of the liver, which is unable to excrete the excess bilirubin. The young age is also responsible for the hepatic hematopoiesis seen [738].

The hemolysis of the fetal red cells is the result of maternal antibodies which have passed through the placenta. Most frequently they are Rh antibodies in an Rh-negative mother who has been sensitized by fetal red cells entering the maternal circulation. Not all Rh-negative mothers produce sufficient antibodies to damage the child, possibly because not enough fetal red cells enter the maternal circulation. Moreover, prolonged or repeated sensitization with the weak Rh antigen is required, and therefore a first pregnancy is usually uneventful, except if the mother has been sensitized by blood transfusions. Incompatibilities in the ABO blood groups are probably more frequent than those produced by Rh incompatibility but the manifestations are usually milder.

If the antibody titer is very high, the fetus is stillborn with hydrops fetalis. With low antibody titers, mild forms develop, showing only moderate anemia and slightly increased indirect-reacting serum bilirubin. When antibody titers are moderately elevated, jaundice that develops within the first 24 hours may become dangerous (icterus gravis). In this form of erythroblastosis fetalis, the

hepatic involvement is significant, and also energetic therapy with exchange transfusions is most important to prevent kernicterus (see Kernicterus, under Influence of the Liver on the Brain, Chap. 53).

In addition to edema, the anoxia resulting from the hemolytic anemia may produce necrosis of hepatic cells, with an inflammatory response potentially leading to fibrosis and cirrhosis. Moreover, the antigen-antibody reaction responsible for the hemolysis may also damage the hepatic cells directly, as suggested by animal experiments [2611].

The structural changes do not vary with the different causes of erythroblastosis; livers in instances of Rh incompatibility between mother and fetus appear similar to those in instances of incompatibilities in major blood groups [1344, 2612, 3733]. Alterations are occasionally found which are related to blood transfusions, especially exchange transfusions. In exceptional instances, massive necrosis, even with calcification, is caused by some substances used in the blood preservation [2818].

The jaundice usually lasts for several weeks, and the children usually have splenomegaly. Liver function is generally normal except for Bromsulphalein retention [3417]. The serum bilirubin increases more rapidly than in physiologic jaundice of the newborn. The serum bilirubin reaches 10 mg per 100 ml within the first 24 hours, and jaundice appears at that time [1564]. Icteric amniotic fluid and a low hemoglobin content in the cord blood, especially with a positive Coombs' test result, are of earlier and greater diagnostic value. The appearance of antibodies in maternal blood is an important alarm signal.

Macroscopic Appearance. Grossly, the liver is usually enlarged, the capsule is smooth, and the color is purple with a green hue. The markings are indistinct, as they usually are in the neonatal liver, and the consistency is not altered.

Histologic Findings. Three main histologic features are noted, viz., excessive blood formation, jaundice, and anoxic injury [678, 1174, 2664]. In addition, fibrosis is found in more protracted cases. The differentiation from simple immaturity, syphilis, and other conditions with increased hematopoiesis is often difficult. The histologic alterations depend on the stage of the disease, and jaundice and erythropoiesis are most severe in infants dying within 2 days after birth. In children dying after several weeks, the changes are less

conspicuous and the hematopoietic foci have disappeared [2664].

EXCESSIVE ERYTHROPOIESIS. An excessive number of erythropoietic cells are seen within the sinusoids, in cellular foci which are free of connective tissue fibers, and irregularly distributed throughout the lobule (Fig. 160, lower right). The circulating red cells, as well as the cells in such foci, are proerythroblasts, but gradually maturation occurs, until erythroblasts, normoblasts, and finally adult red cells are mixed with the blast elements. The cells in any one focus are said to show the same stage of maturation in erythroblastosis, in contrast to other conditions with increased hepatic erythropoiesis [2664]. These erythropoietic foci sometimes encroach upon the hepatic cells, and the hepatic-cell plates are broken into small groups of cells. The portal tracts are much enlarged and are very cellular, most of the cells being myelocytic rather than erythrocytic, while eosinophils may be present.

JAUNDICE. Occasionally the hepatic cells of still-born infants with erythroblastosis contain bile pigment, probably an indication of impaired clearance by the immature liver. Usually increased bile pigment deposition appears after 2 days of life. At this time the hepatic cells contain a granular pigment and often iron pigment as well. They surround dilated bile canaliculi with many bile thrombi. Many of the hepatic cells reveal fat vacuoles and coagulated protein. The Kupffer cells are prominent and often contain brown pigment as well as iron pigment after several days.

TOXIC CHANGES. The toxic changes, especially mid-zonal necrosis, found in 10 of 141 cases [1476], are most severe in children living more than 10 days. The necrotic areas vary in size from a few cells to destruction of approximately one-fifth of a lobule. They are irregularly distributed but are usually mid-zonal. The hepatic cells frequently contain fat, and many of them are anuclear or reveal coagulation necrosis. Some cells have disappeared and are replaced by segmented leukocytes.

FIBROSIS. The framework is rarely collapsed in places; in the few cases associated with collapse, fibroblasts have been described, suggesting new formation of fibers. In the vicinity of these collapsed or fibrotic areas in the lobular parenchyma, connective tissue membranes are noted, often separating individual cells. At the same time the portal tracts appear enlarged, and perilobular fibrosis and ductular proliferation are frequent.

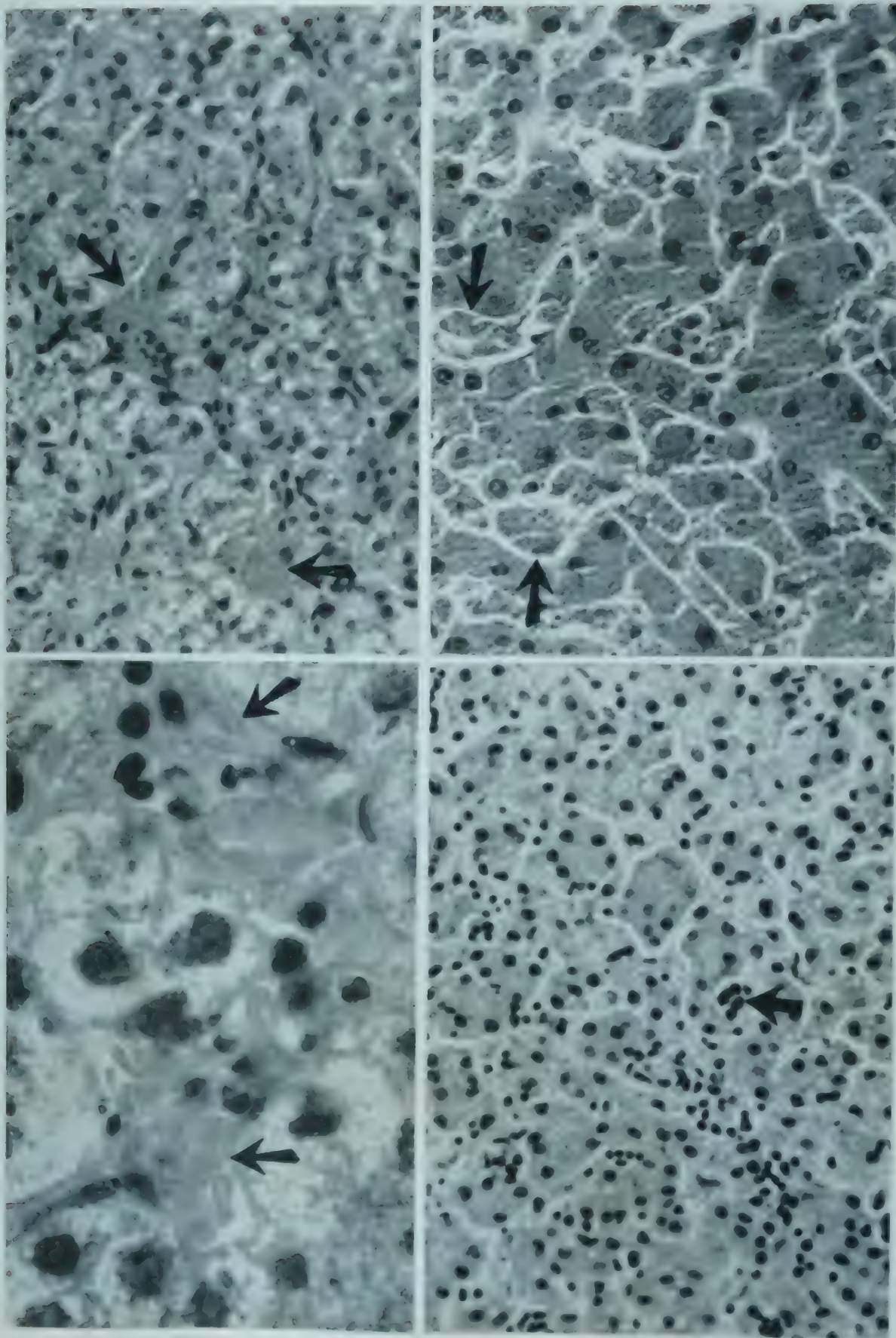


FIG. 160. *Upper left.* Biopsy specimen revealing sickling. The sinusoids throughout the lobule are crowded by crescent-shaped red cells in rouleau arrangement (arrows). H&E ($\times 240$). *Upper right.* Autopsy specimen of patient dying in sickle-cell crisis. The sinusoids are uniformly dilated by sickling red cells (arrows), and the arrangement of the hepatic-cell plates is disturbed. H&E ($\times 240$). *Lower left.* Higher magnification of upper right. Crescent-shaped red cell in dilated sinusoids (arrows). ($\times 660$). *Lower right.* Erythroblastosis fetalis. In and around the sinusoid many hepatocytic foci (arrow) are noted containing immature red cells with large nuclei. H&E ($\times 240$).

Regeneration is active, especially on the lobular periphery. These instances of intralobular and perilobular fibrosis in erythroblastosis fetalis [1172, 2664] are usually found in infants dying after 2 weeks of life, but occasionally they are encountered even in stillborn or macerated fetuses [1457]. They are the intermediate stage in the development of the rare instances of cirrhosis in the neonatal period supposedly caused by erythroblastosis [678]. In such instances, diffuse intralobular necrosis and fibrosis predominate, but the presence of a few regenerative nodules and portohepatic vascular anastomoses permit the use of the term "cirrhosis."

Sickle-cell Anemia

In the form known as sickle-cell disease all the hemoglobin is abnormal, while in sickle-cell trait part of it is. The sickle hemoglobin crystallizes in erythrocytes if the oxygen tension is reduced. This results in alteration of the shape of the red blood cell, or sickling, which increases the viscosity of the blood and leads to conglutination of cells, with thrombus formation and vascular occlusion. The life-span of the erythrocytes is shorter than normal, and hemolysis develops in crises. Sickle-cell trait produces no hepatic changes during life, and the sickling of red cells in the sinusoids found at autopsy develops in the agonal period. Sickle-cell disease causes significant hepatic changes, many of which are similar to those of any hemolytic anemia. In sickle-cell crisis, disturbed blood flow with or without obstruction is the main hepatic alteration. The sinusoidal blood flow is impaired by conglutinated sickled erythrocytes and enlarged Kupffer cells containing engulfed red cells [1264].

Clinical Manifestations. In sickle-cell disease some degree of jaundice is present in approximately 20 per cent of cases. If the serum-bilirubin level increases above 4.0 mg per 100 ml, associated liver damage has to be assumed. Serum-protein changes, such as reduction of albumin or increase in gamma globulin, are common [993]. In sickle-cell crisis, the liver is enlarged and tender. It rapidly returns to normal size following the crisis [1302, 1455], which fact results in discrepancies in published reports regarding liver size [2217]. Some jaundice is found in more than half of the cases during a crisis [1455]. The elevated level of prompt-reacting bilirubin in the serum

found in the majority of the cases indicates hepatic injury. Urinary urobilinogen is increased, partly because of hemolysis and partly because of associated hepatocellular injury [2217]. In rare instances severe jaundice develops rapidly, as in fulminant hepatitis, and the question of serum hepatitis arises. However, the hepatic-cell damage in sickle-cell crisis may be severe enough in the presence of hemolysis to explain this steep rise in the serum-bilirubin level. In jaundiced patients total serum cholesterol is increased and cephalin flocculation is often increased, while serum-alkaline phosphatase activity is not [1455]. In nonjaundiced patients abnormal results of hepatic tests are unusual, except for Bromsulphalein retention.

Structural Alterations. In sickle-cell anemia, the Kupffer cells are characteristically enlarged and contain lipofuscin and iron pigment as well as erythrocytes. Phagocytosis is occasionally the outstanding histologic feature in the liver [1264, 3171]. Hepatic-cell changes, i.e., focal necrosis, fatty metamorphosis, and pigmentation, are frequent [181, 795]. Sickling may be noted in biopsy specimens (Fig. 160, upper left). Cholelithiasis is common (see Gallstone Formation, Chap. 30).

In patients dying in sickle-cell crisis, the liver is enlarged and has a purple-bluish hue and a tense, smooth capsule. The lobular markings are not clearly discerned. Histologically, the capillaries are overdistended by packed erythrocytes, most of which are crescent-shaped [1264] (Fig. 160, upper right and lower left). In contrast to passive congestion, this distention is the same in the peripheral portion of the lobule and in the center. The hepatic-cell plates are compressed and sometimes broken, and the hepatic cells show various degrees of degenerative changes [795, 1264]. In the instances with severe jaundice, extensive hepatic necrosis, mainly centrilobular, can be noted, as well as focal parenchymal alterations [329]. Transitions into cirrhosis have been observed [3135]. Occasional hepatic necrosis in sickling, with areas of infarction elsewhere in the body, indicates that vascular obstruction may precede death by a sufficient length of time to produce ischemic necrosis [1752]. Hepatic arterial thrombosis has also been observed, as well as portal vein thrombosis and ischemic necrosis of the liver without demonstrable obstruction [1752]. Secondary hemochromatosis has been seen [329].

NUTRITIONAL HEPATIC INJURY: EXPERIMENTAL AND ETIOLOGIC CONSIDERATIONS

Hepatic function and structure depend upon the metabolic status of the organism and thus also upon the availability of many metabolites in the liver. For instance, meals increase the weight of the liver about 20 per cent within 4 hours, mainly because of glycogen deposition; the organ returns to its original weight in 12 hours [334]. The liver is the target of deficiencies, excesses, and especially imbalances [2640] in available metabolites. Hepatic alterations produced by deficiencies in each of several nutrients have been demonstrated in various animal experiments [1193, 1320, 2202, 2752, 2969, 3150, 3534]. Intake of nutrients insufficient to fulfill increased needs in various circumstances, such as pregnancy, represents a relative nutritional deficiency. This type of deficiency is now recognized as the main cause of the hepatic injury produced by alcohol abuse. Injuries produced by chemical toxins and prevented by nutrients may be examples of a similar relative deficiency [833, 949]. In the presence of normal nutritional intake, processes in the body, such as destruction or excretion of nutrients, as well as biological antagonism to them, may create endogenous or conditioned deficiencies, such as have been described for vitamins [1649] and amino acids [1823]. In clinical medicine deficiencies of single or multiple nutrients play a great role as complicating factors in many hepatic diseases. In contrast to variations in the nutritional lesions produced in experimental animals, hepatic changes in man owing purely to dietary disturbances, at least in the Temperate Zone, are largely restricted to brown atrophy, in simple starvation, and to the fatty liver with its sequelae, in nutritional imbalance.

Little is known about changes in hepatic func-

tion and structure in man from deficiencies of single nutrients, such as protein, in the absence of such complicating factors as disease, general undernutrition, or malnutrition. Only a few human experimental studies are available. Diets producing fatty livers in rats caused enlargement of the liver, Bromsulphalein retention, and abnormal blood lactate and pyruvate levels [1690]. The effects of experimental diets upon the disappearance of nutritional damage in alcoholic persons have been studied [2587].

Influence of Physiologic Dietary Variations

DIURNAL VARIATIONS. Glycogen and bile pigment distribution in the liver lobule undergo cyclic variations during the day [752], probably originally determined by the pattern of meals. In this diurnal rhythm, deposition of glycogen following meals begins initially with small amounts on the periphery [3010] and is quickly followed by massive deposition in the center of the lobule. It spreads toward the periphery, from which glycogen subsequently disappears first. The glycogen depots are greatest in the early morning hours. Bilirubin formation is the opposite of glycogen formation as far as lobular topography and variations are concerned, bilirubin secretion being lowest in the early morning hours [3038].

DIETARY VARIATIONS. In addition to these diurnal variations, several meals richer in one or the other of its constituents but still not considered unbalanced influence hepatic function and structure. For instance, after meals relatively rich in carbohydrates, the glycogen content of the liver is very high. After a high-protein diet, the cytoplasm of the hepatic cells contains many basophilic granules. A relatively high fat intake does not increase

the visible fat stores as long as the intake is not excessive. Any cytologic evaluation of the liver should take such diurnal or alimentary physiologic variations into consideration.

EFFECT ON HEPATIC TESTS. Physiologic variations of the diet affect the results of hepatic tests. Galactose tolerance is influenced by diet, because hepatic glycogen and fat stores determine the formation and deposition of glycogen. Hippuric acid formation depends on an adequate intake of glycine [173]. The administration of glucose improves hippuric acid synthesis and glucuronate formation impaired by damage to the hepatic parenchyma [3112].

NUTRITIONAL HEPATIC INJURY IN ANIMALS

In animals, three mechanisms of nutritional hepatic injury can be clearly demonstrated, in addition to the changes produced by physiologic variations in the diet. These are (1) starvation or caloric undernutrition; (2) specific deficiencies of a dietary constituent; (3) imbalance of various food constituents with and without alteration of the caloric intake.

Starvation

In starvation, or chronic undernutrition, the body lives by reducing the metabolic level and by utilizing its own resources, including body glycogen, fat, and protein. It thus makes available the necessary metabolites in a balanced relation. The structural alterations are mainly those of reduction of the size of the organ and of depot substances. As starvation proceeds, mobilization of peripheral depots again increases some hepatic depots. Hepatic glycogen decreases during fasting in all species studied within the first 48 hours, whereas subsequently storage of glycogen has been reported [1736]. Starvation generally reduces the fat content [260]. However, in mice, liver fat increases parallel with a reduction of phospholipids during early stages of starvation, so that its absolute amount is higher despite reduction in the size of the organ [2145]. In rats, this increase of fat is not noted [3357], and reduction of fat is greater in males than in females. In man, liver fat is usually low (see Lipid Metabolism, Chap. 5). During fasting the liver adds ketone bodies to the blood [683]. Hepatic proteins decrease, as do pentose nucleic acids, while desoxypentose nucleic acids remain unchanged [477, 735, 1898], and the

DNA/PNA ratio increases [653]. Hepatic and serum cholinesterase are reduced [1399].

Nutritional Deficiencies

The evaluation of the effect of deficiencies of the major nutrients is not simple, because deficiency of one usually occurs with an excess of another, and therefore many experiments with deficiency of major nutrients represent imbalance, rather than deficiency of a single substance. Moreover, the total caloric intake determines the effect of deficiency. It is exaggerated by high caloric intake and attenuated by low caloric intake, which retards metabolism and growth. Similarly, during periods of growth any deficiency becomes more apparent. With these reservations, deficiency of individual substances will be listed, followed by a description of obvious imbalances, excesses, and combinations of deficiencies.

Types of Protein Deficiency. Deficiency of dietary protein is either a deficiency in total protein, independent of the constituent amino acids, or deficiency of certain amino acids which have specific functions in the liver. Other unknown factors may be present, because fatty livers develop in rats on purified diets containing all known essential amino acids [2808].

Deficiency of Total Protein. Reduced protein intake or reduced protein absorption owing to intestinal or pancreatic defects [1410, 2884] results in a reduction of the size of the lobules and cells [1618], with a decrease of the stainable protein in the hepatic cells and disappearance of the basophilic cytoplasmic pentose nucleic acid [1844, 1898]. Low-protein diet also produces fatty liver [529, 706, 1320, 1990, 2752, 3459]. This fatty liver is associated with early hepatic-cell degeneration and focal necrosis [1821]. Although the hepatic cells appear vacuolated, this is caused not only by fat or glycogen [922, 3481] (Fig. 161, lower left). Nucleoli are enlarged, and basophilic material accumulates around the nucleus, probably indicating attempts at pentose nucleic acid formation [3239]. Various types of protein-deficient diets produce zonal or massive necrosis with diffuse fibrosis, or mixtures of necrosis and fatty liver [320, 706, 707, 1320, 1372, 1498, 1804, 3459, 3526]. The fatty liver with subsequent fibrosis is probably the result of combined deficiency and imbalance [707, 1348, 1372, 1498] (see Hepatic Injury from Dietary Imbalance or Multiple Factors, later in this chapter).

The total nitrogen of the liver is decreased [922,

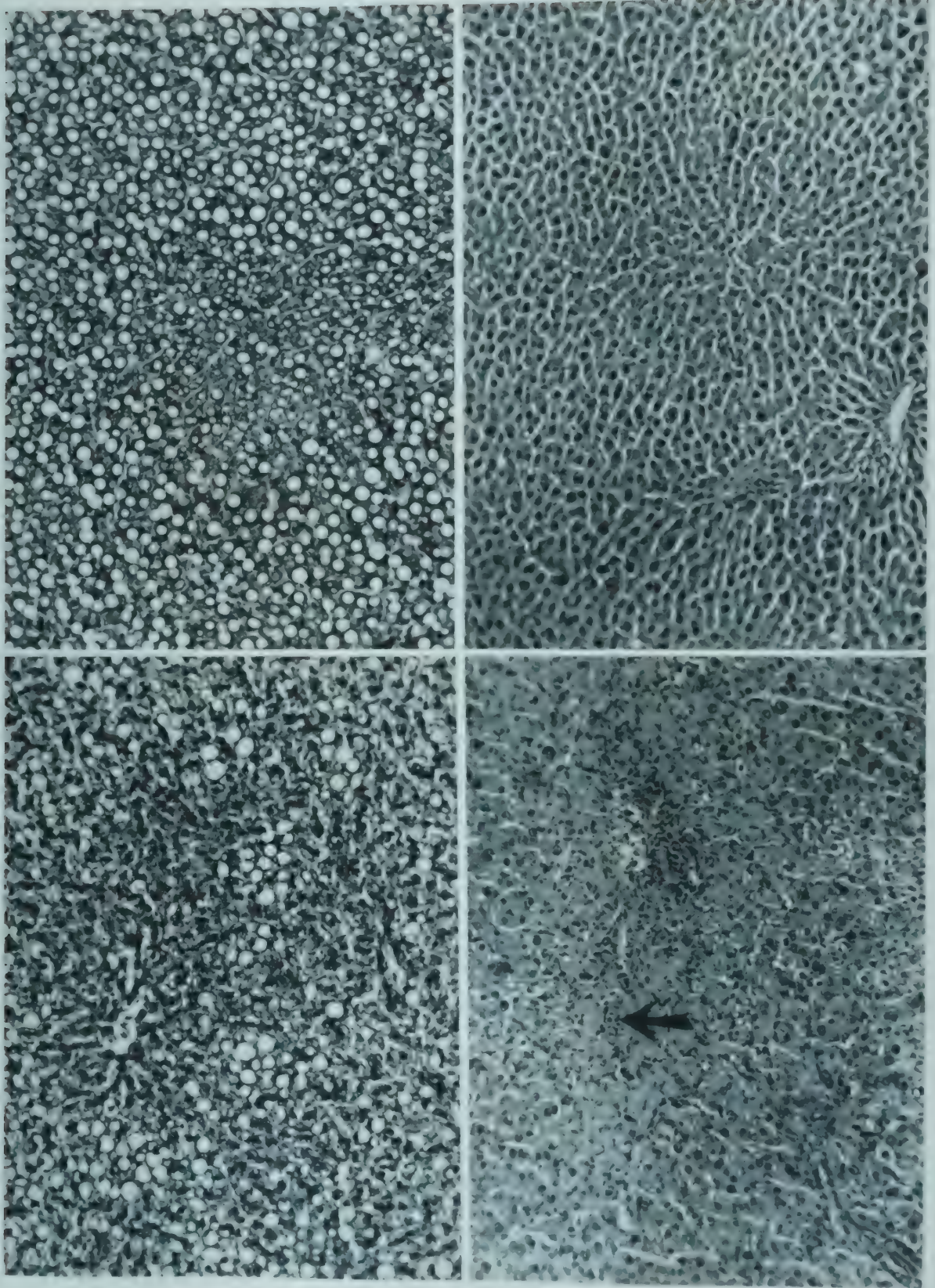


FIG. 161 Photomicrographs of rat livers. H&E ($\times 120$). *Upper left.* Choline-deficient diet for 5 weeks. The hepatic cells contain large fat droplets; they reveal no other evidence of hepatic-cell damage. *Upper right.* Choline-deficient diet for 5 weeks, then 15 mg choline orally 48 and 32 hours before death. The hepatic cells are normal except for few scattered fat droplets. *Lower left.* High-fat-low-protein diet for 4 weeks. The hepatic cells contain many large fat droplets. The cytoplasm of the fat-free hepatic cells is irregularly clumped. Where hepatic cells are missing, scavenger cells accumulate (focal necrosis). *Lower right.* High-fat-low-protein diet for 4 weeks, then 15 mg choline orally 48 and 32 hours before death. The hepatic cells are almost free of fat. The hepatic-cell plates are irregularly arranged. Cytoplasm and nuclei of hepatic cells vary in staining qualities. The former is clumped in places, and small areas of focal necrosis are noted (arrow).

923, 1618, 2382, 3481], as is the total protein [1844, 1898, 2294, 2827]. Pentose nucleic acid (PNA) is reduced, while desoxypentose nucleic acid (DNA) apparently increases [477]; this, however, has not been uniformly found [2382]. The hepatic nitrogen/DNA ratio has been used as an index of the state of nutrition. The lipid phosphorus decreases, together with an increased turnover of the remaining lipid and PNA phosphorus [477]. Reduction of riboflavin [2382] and water [1618] and general redistribution of the intracellular components occur [2382]. The fat content, as determined chemically, generally increases [269, 1172, 1348, 1618, 1823, 3481], although this depends upon the experimental conditions, and sometimes fat again disappears with prolonged protein depletion.

Many hepatic enzyme activities are reduced, including those of esterase [2257, 2637], xanthine oxidase [893, 2257, 2294], and catalase and arginase [369], but alkaline phosphatase activity increases [2637, 2827], although not uniformly [2294]. Liver glutathione is reduced [893]. Bromsulphalein retention is increased [1821, 3481].

Deficiency of Specific Amino Acids. Deficiency of one of the essential amino acids interferes with protein formation and leads to hepatic changes, even if the total nitrogen in the diet is not significantly reduced. Such deficiencies may result from the intake of various vegetable proteins which are lacking in some of the essential amino acids. Fatty metamorphosis, apparently similar in appearance, has been reported from deficiencies of various amino acids, even including nonessential ones such as threonine or lysine [352, 787], tryptophane [15], and leucine [2247]. In tryptophane deficiency, xanthine oxidase activity is reduced while hepatic protein remains normal [3606]. Leucine deficiency causes some fatty metamorphosis in the liver, while phenylalanine or histidine deficiencies do not [2247].

Two amino acids, methionine and cystine, appear to be of specific importance in hepatic metabolism. Growth and general metabolic stimulation as a result of the specific dynamic action of protein in a high-protein or high-caloric diet increase the need for these amino acids and thus aggravate the deficiency.

METHIONINE DEFICIENCY. Methionine deficiency becomes manifest as (1) a deficiency of an essential amino acid; (2) a sulfhydryl deficiency; (3) a methyl donor deficiency. Uncomplicated methionine deficiency produces a fatty liver [3370] but

not necrosis. The amount of methionine available for removal of fat is limited by that needed for growth, and the requirements depend on the amount needed for growth and for methyl donors [3356]. Methionine prevents or attenuates various hepatic injuries produced by poisons. Methionine is specific in the correction of a conditioned deficiency such as results from ethionine administration [2635]. In other intoxications, such as that by carbon tetrachloride, chloroform, selenium, trinitrotoluene, or arsphenamine, the protective mechanism is not so well established [833, 949] and is mainly apparent in protein-deficient animals. The protective effect of protein in these intoxications largely results from its methionine content, and this effect is also more apparent in protein-depleted animals [151]. The inhibition by methionine of tumor formation from azo dyes has been related to improved retention of riboflavin [1280]. Methionine deficiency reduces hepatic xanthine oxidase and succinic dehydrogenase [3605].

ETHIONINE ADMINISTRATION. The administration of ethionine analogue and probably a biologic antagonist of methionine produces a conditioned methionine deficiency. Administration of single doses results in fatty livers in female rats (Fig. 131C) but not in males [976]. This is associated with interstitial pancreatitis. Prolonged administration produces hepatocellular degeneration and regeneration in both sexes, with central necrosis, proliferation of ductular cells, interstitial infiltration with mesenchymal cells, and diffuse increase of reticulum fibers (Fig. 132A). This is accompanied by pancreatic atrophy and fibrosis. Repeated focal necrosis eventually leads to post-necrotic cirrhosis (see Collapse, or Postnecrotic Cirrhosis, Chap. 28) and subsequent formation of tumorlike nodules consisting of either hepatic cells or bile ducts (cholangiofibrosis) [2635] (Figs. 191, 192) (see Hepatocellular Nodule, also Bile Ductal and Ductular Nodule, under Histology of Experimental Hepatic Neoplasms, Chap. 58). Morphologically, the lesions produced by ethionine in rats resemble the changes in human tropical malnutrition (see Juvenile Kwashiorkor, also Adult Kwashiorkor, under Tropical Malnutrition, Chap. 51). The mechanism of the ethionine injury is not established; a blocking effect of protein synthesis has been claimed, but it is more likely that ethionine interferes with choline synthesis and decreases choline oxidation [3198, 3278]. Ethionine therefore is an antimetabolite to choline as well as to methionine [3197].

CYSTINE DEFICIENCY. A diet deficient in cystine produces hemorrhagic zonal or massive necrosis in the liver [3534]. On cystine-deficient diets with borderline doses of methionine to prevent anemia and hypoproteinemia, massive necrosis occurs [1497]. The cystine effect is most noticeable in relation to other alterations. For instance, cystine deficiency delays the formation of hepatic tumors [3584].

Various Excesses and Deficiencies. **LOW-FAT AND LOW-CARBOHYDRATE DIETS.** Structural changes from deficiency of carbohydrates or fats are the result of imbalances or excesses.

EXCESS CARBOHYDRATE INTAKE. Excess carbohydrates produce fatty liver by raising the caloric intake and the demand for choline and other factors [259]. Parenteral administration of large amounts of glucose leads to bilirubinemia and dye retention, apparently related to excessive glycogen storage in the liver [334]. Liver arginase is reduced on a high-carbohydrate diet [1817].

EXCESS FAT INTAKE. Excess fat in the diet of rats produces fatty livers, which are prevented by the administration of choline [543, 777]. This fatty metamorphosis is exaggerated by a low protein content in the diet. Moderate amounts of fat are beneficial and do not interfere with regeneration [2799]. Increased requirement for choline, producing a relative choline deficiency, probably explains this lesion. The type of excess fat and fatty acids in the diet is important in the development of the fatty liver [471]. Feeding of large amounts of cholesterol to various animals produces hypercholesteremia and fatty liver. The liver contains much cholesterol, which disappears slowly after choline administration [258, 261]. This is probably caused by overtaking of the lipid-removal mechanism, in that cholesterol competes with phospholipids for unsaturated fatty acids [2572]. The addition of cholic acid increases cholesterol deposition, while dihydrocholesterol decreases it [206].

EXCESS PROTEIN INTAKE. Excessive protein intake has been considered harmful to the liver because of the increased demands for deamination. In the animal with an Eck fistula, the feeding of meat results in meat intoxication (see Eck Fistula, under Portal Vein, Chap. 18) associated with further increases of serum alkaline phosphatase and reduction of dye clearance [1088]. Feeding of excess cystine produces portal necrosis and hemorrhages in the liver, eventually resulting in cirrhosis [873]. This is modified by variations in the

protein and fat content of the diet but not by choline. Excess cystine facilitates the deposition of fat [1281], but the cirrhosis is not necessarily related to this. Moreover, even small amounts of cystine increase the tendency for cirrhosis formation, although cystine assists in preventing massive necrosis [1318]. Small doses of cystine cause a nonspecific increase in the metabolic needs, with a relative deficiency of methionine or choline [1542]; the toxic effect of large doses is unexplained [3355].

HEPATIC INJURY FROM DIETARY IMBALANCE OR MULTIPLE FACTORS

Most nutritional injuries fall into this category, including the lesions resulting from (1) low-protein and high-fat diets; (2) deficiency of several antinecrogenic factors; (3) alcohol. The analysis of functional and structural changes produced by these complex nutritional disturbances is made difficult by great variations in experimental conditions and by species and age differences.

Lipogenic-Lipotropic Imbalance: Experimental Fatty Liver-Cirrhosis Syndrome

The complex relation of the various nutritional factors is best understood as a disturbance of a balance between various lipogenic and antilipogenic factors, each with a different weight [2640] (Fig. 162). Increase in one factor, such as fat, may produce no change if another factor, such as protein, is similarly increased. Imbalance results in the accumulation of fat in the liver often from exogenous sources [1990], eventually followed by fibrosis and cirrhosis. Glycogen deposition may simultaneously be increased [3357].

Pathogenesis. **LIPOGENIC FACTORS.** A diet with less than 10 per cent protein and more than 40 per cent fat and containing less than 2.0 mg choline per day produces a fatty liver after several weeks in adult rats or after a few days in weanling rats [320, 706, 1172, 1320, 1348, 1498, 3526]. Eventually cirrhosis develops. High-fat diets with 16 per cent protein produce the same lesion in dogs [530], as does a low-fat diet with 6 per cent protein [1348]. On a diet in which all other components are fixed, the hepatic changes depend on the amount of dietary fat or dietary choline [1194], but a high fat content is not necessary if the intake of lipotropic substances is kept low [706]. Among the fats, lard appears to be more lipogenic than vegetable shortenings, and cod-liver

il is apparently even more so. Incomplete proteins facilitate the development of fatty liver and cirrhosis. Fatty liver can be produced on a relatively high-protein diet if it is low in methionine and choline. Peanut meal is the source of protein on such a diet [655, 1408]. The same is true if cornmeal is used in attempts to duplicate the diets of persons with kwashiorkor [3048]. In growing animals the lesion develops faster [1281, 1373], and stress in general also accelerates its development [1968]. Riboflavin and niacin [1864] deficiencies produce it also, whereas vitamin A is without effect [1320].

LIPOTROPIC FACTORS. Methionine and choline prevent or arrest the development of the fatty liver [707, 1320, 1619], although a high-protein diet without choline supplements is superior to a low-protein diet supplemented with choline or methionine for repair of fully developed lesions [1320, 1607]. The lipotropic effect of protein was originally related to methionine [3370], but casein has more lipotropic action than its content of methionine would lead one to expect [258, 261]. Therefore, protein, independent of its methionine content, contributes to fat removal by providing con-

stituents for fat-oxidizing enzymes. However, the finding of a decrease in activity of one enzyme in the presence of protein deficiency does not imply that loss of this enzyme is responsible for the fat accumulation. Liver extract [836], pyridoxine [1318], and vitamin B₁₂ [1322] prevent fatty liver, while brewer's yeast gives varying results [836]. Reduction of the metabolic needs by starvation [260], by administration of thiouracil [1323], or by exposure to cold [3001] slows the development of the fatty liver-cirrhosis syndrome, whereas metabolic needs increased by cystine [707, 1320], thiamine, or biotin [1138, 2125] aggravate it. Male rats are more susceptible than females [1320], and estrogens have a protective effect [1324]. Antibiotics, especially tetracyclenes, suppress cirrhosis formation despite stimulation of growth [1327]; whether this is an antibacterial effect on the intestinal flora, or whether it spares essential nutrients such as methionine is unknown [1318].

Functional Manifestations. In rats, ascites and pleural and pericardial effusions, sometimes bloody or chylous, are found, probably related to hypoproteinemia [1320]. Bromsulphalein retention occurs early, while serum and hepatic alkaline phos-

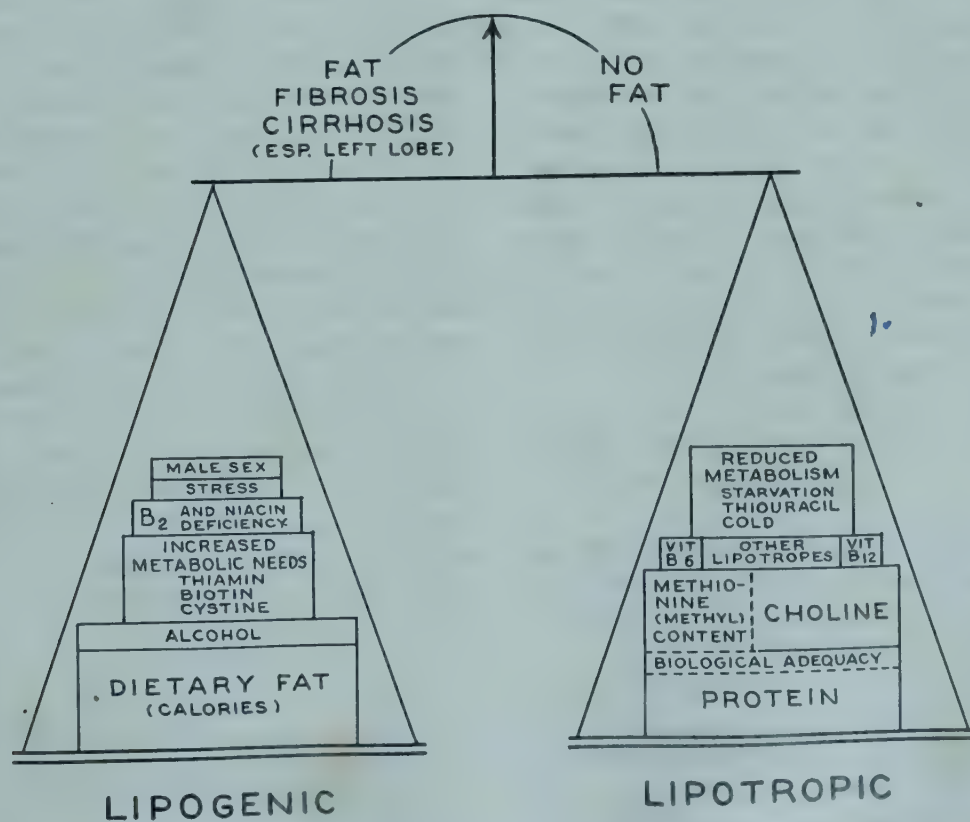


FIG. 162 Balance between lipogenic and lipotropic substances, its disturbance leading to the fatty liver-cirrhosis syndrome. (Popper, H., and Schaffner, F.: *A.M.A.Arch.Int.Med.* 94:785, 1954.)

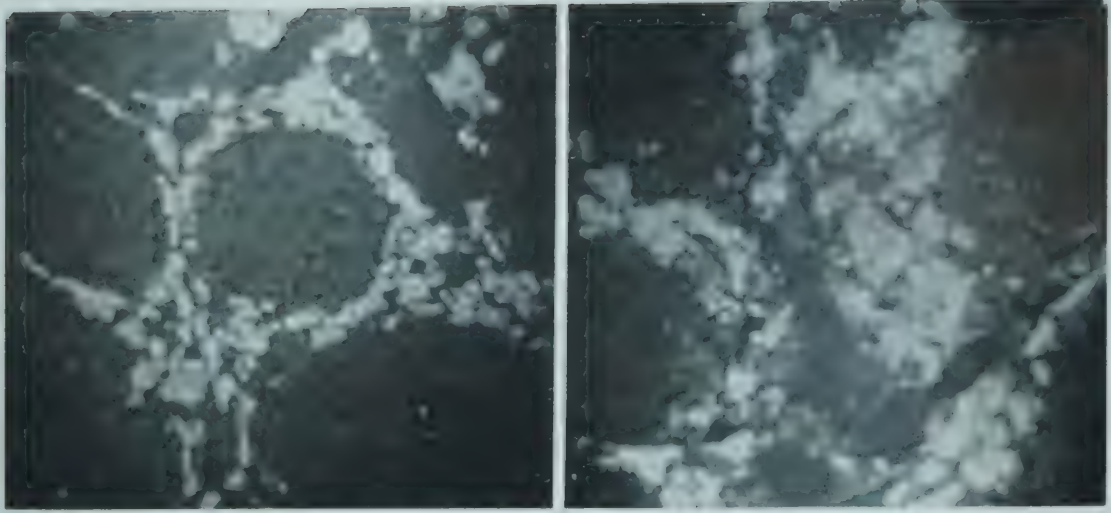


FIG. 163 Fluorescence photomicrographs of unstained sections. Ceroid in macrophages of livers of rat with experimental nutritional cirrhosis. *Left.* Ceroid fluorescence outlining the septums ($\times 60$). *Right.* Different degree of ceroid fluorescence in the same deposition ($\times 150$).

phatase increase later, with reduction of serum and hepatic esterase [839, 1821]. Depression of hepatic dye excretion has also been noted in dogs [1990]. The tolerance of the animals for anesthesia and minor surgical procedures such as biopsies is poor [1320], but the regenerative ability of the liver is not altered [3609]. Removal of fat by choline does not reduce the functional or structural signs of hepatocellular degeneration [1821] (Fig. 161, upper right and lower right).

Structural Changes. The earlier changes are fatty infiltration, usually more severe in the center of the lobule, with the eventual development of fatty cysts [1405, 1406, 1407, 1408]. Choline deficiency differs from protein deficiency in that functional and structural evidence of hepatocellular degeneration exists in the latter (Fig. 161, upper left and lower left). After several months fibrosis and cirrhosis develop, characterized by central or, at least, nonportal connective tissue accumulation [110, 1406, 3609], more severe in the left lobe [1172]. In rats the fibrosis and cirrhosis directly result from fatty infiltration [1407, 1408, 1497]. Some believe that hepatic necrosis is required [1318, 3459], pointing to necrotic cells in and around the septums [655, 1320, 1372] and emphasizing the long interval between maximal fatty infiltration and fibrosis [1372].

CEROID. In the liver and other organs, especially the spleen and lymph nodes, of rats on choline- or methionine-deficient diets, an orange-brown granular pigment called "ceroid" is noted [706, 933, 1320, 2633]. It is insoluble in lipid solvents and gives a sudan reaction and a specific color reaction with basic aniline dyes, especially methyl

green, even in paraffin sections [320, 1320, 2003]. Ceroid is strongly brown fluorescent except when it is concentrated; it is dark brown in visible light [2633] (Fig. 163). In the liver, it is found mainly in large mesenchymal cells in the developing septums of rat cirrhosis, particularly in the centrolobular region. Ceroid is also found in the livers of rats with cirrhosis from carbon tetrachloride and in cirrhotic mice, although rat ceroid seems to differ histochemically from mouse ceroid [1931]. Similarities with uterine and ovarian pigments in vitamin E deficiency have been claimed [2521].

Recent fluorescence studies and examination of acid-fast stains contradict previous claims that ceroid is not found in man [1405, 2003, 2633]. In human cirrhosis it has occasionally been seen [2521], and in hepatitis and other types of necrosis, such as in tuberculosis, ceroid-containing cells have been described [2940].

Ceroid was originally thought to be a pigment in fat which was concentrated after disappearance of the fat from the rest of the cell [2633]. Ceroid was also linked to unsaturated fatty acids [933]. Reduction of cod-liver oil in the diet [333, 932] or addition of tocopherol [1318, 3423] reduces ceroid deposition. Recently, ceroidlike substances have been produced in experimental hematomas in the mesentery and also in vitro by mixing cod-liver oil and red blood cells [1405]. This suggested that ceroid develops from erythrocytes hemolyzed by fat in the fatty cysts of the cirrhotic liver in the absence of nutritional substances, possibly tocopherol, which prevent the normal disintegration of hemoglobin to hemosiderin. Additional histochemical studies led to the conclusion that

ceroid and the pigment of vitamin E deficiency develop by autooxidation of fats if biologic anti-oxidation is prevented by excessive accumulation of unsaturated fatty acids [514].

Dietary Necrosis

In early nutritional experiments, necrosis was found in rats in which fatty liver with fibrosis and cirrhosis was produced [706, 1320]. No sharp differentiation between necrosis and the fatty liver-cirrhosis syndrome was made, mainly because of the erratic occurrence and distribution of the necrosis. The lack of the response of the necrosis to choline suggested that it was an independent lesion [707]. Gradually a combination of factors was recognized as the cause of massive necrosis [1193, 1320, 1497, 1498]. These findings were in accord with earlier German investigations [1512, 2968, 2969]. This lesion differs from the fatty liver progressing to cirrhosis produced by high-fat-low-protein diets in its histologic appearance; it is not the result of an imbalance, and it appears suddenly with no premonitory clinical manifestations and few preceding morphologic changes.

Etiologic Factors. For the production of dietary necrosis in rats, deficiency of three factors is required, sufficient amounts of any one preventing the lesion. These factors are (1) cystine or its precursor, methionine [3, 770, 1318], the main factor in the necrogenic effect of the low-protein diet; (2) alpha-tocopherol [3, 1320, 2008, 2968, 3006]; (3) an unknown factor found in American yeast and thus far unidentified [2968, 2969]. This complex situation of deficiencies of three factors explains some of the difficulties in animal experiments. Partially denatured casein [2968] or certain yeasts serve as a base for the necrogenic diet. Other yeasts are not necrogenic [1325]. Either factor 3 deficiency [2969] or toxicity [1325] accounts for the differences in species of yeast. Antibiotics delay the development of necrosis [3, 1327, 1328], suggesting that intestinal bacterial products incompletely detoxified, or destruction of protective substances by bacteria, are responsible for the lesion. Male rats and younger rats are more susceptible [3, 1373]. The greater susceptibility of growing rats is a reflection of increased metabolic needs, since thyroid supplementation increases the necrogenic effect and propylthiouracil reduces it [3]. High temperature aggravates the lesion [2404]. A high-fat diet, choline, or vitamin B₁₂ has no effect [3, 1322] or sometimes is slightly

injurious [1318]. Fat-free diets delay the development of necrosis, possibly by altering the intestinal flora, somewhat as antibiotics do [2151].

Functional Changes. Rats on a necrogenic diet appear perfectly normal and even gain weight until a few hours before death [3, 1497, 1523]. Suddenly they become quiet and die without convulsions. In this short preterminal period they show hemoconcentration, aminoaciduria, bilirubinuria, and urobilinogenuria. Excretion of ether-soluble acids is increased [1049]. Some animals survive, and after 1 week they again begin to gain weight. Some of these rats remain well, while others succumb to a second attack. In a few, jaundice and ascites develop, as evidence of chronic hepatic failure.

Structural Alterations. ACUTE NECROSIS. In the pre-necrotic stage few changes are noted. Just prior to the development of necrosis, cytoplasmic basophilia decreases. The lipids increase, especially in the centrilobular zone [3]. Massive or submassive necrosis develops suddenly, with merging of the cytoplasm of the hepatic cells into a mass, which is rapidly removed and replaced by scavenger cells. The framework collapses, and massive or submassive collapse ensues. In the acute stage, the protein content is reduced, and the water content is greatly increased, as evidence of hepatic edema. The degree and extent of the changes vary in the different laboratories in which the lesion has been produced [3, 706, 1193, 1320, 1498, 1523, 2969]. The gross and microscopic similarity to the massive and submassive necrosis in human viral hepatitis has been demonstrated [1497]. The more severe involvement of the left lobe in both conditions has been explained by protection of the right lobe by nutrients coming from the intestine.

CHRONIC CHANGES. Postnecrotic cirrhosis with regenerative nodules has been found in surviving animals, especially after repeated episodes of necrosis [3, 1497]. This was not seen in animals protected by tocopherol after the initial attack [1523]. After repeated attacks the water and nitrogen content of the liver is reduced, while fat is increased and glycogen content is unchanged; glutathione and ascorbic acid are reduced. Tocopherol does not prevent these changes, but necrosis does not develop while methionine and cystine are fully protective [2009].

Pathogenesis. The morphologic findings do not aid in the understanding of the pathogenesis of dietary necrosis. Sudden swelling of cells on the periphery of the lobule has been held responsible

for rapid interruption of the lobular blood supply with resulting anoxia [1497]. Involvement of larger vessels is suggested by the lack of uniformity of the lesion in the same liver, in different animals of the same group, and in different laboratories. The sudden development of the lesion in a previously intact organ throws some doubt upon a simple nutritional effect.

Modification of Intoxications by Nutritional Factors

The nutritional status influences the effect of intoxications, especially those affecting the liver. The mechanism is usually one of reduced availability of nutrients or their metabolites for detoxification or other reactions. The intoxication produces a relative or conditioned deficiency which is amenable to nutritional therapy; for instance, prevention of central necrosis from bromobenzene by cystine administration (see Diffuse Hepatic Degeneration, under Experimental Hepatic Injury, Chap. 41).

STARVATION. Starvation aggravates almost all intoxications [833].

HIGH-CARBOHYDRATE DIET. The beneficial effect of a high-carbohydrate diet was one of the first nutritional factors known to influence the course of an intoxication. This has been related to the principle that increased glycogen in the liver protects that organ from injury [334, 833]. Protection offered by a high-carbohydrate diet has been reported in many conditions. Such protection is due either to sparing of hepatic glycogen for the supply of energy needs or, more probably, to sparing of proteins for regeneration of the liver [833]. The conditions affected include acute chloroform [1213], carbon tetrachloride [334, 833], phosphorus, and acute and chronic arsenic [2271, 3442] intoxications. In the evaluation of a high-carbohydrate diet, accompanying nutritional metabolic factors, such as the amount of protein in the diet, are important.

HIGH-FAT DIET. The deleterious effect of excessive fat in the diet has been related to the increased susceptibility of the fatty liver to various toxins [833]. Increased solubility of the poison in the fat of the liver has been held responsible [833]. However, toxic agents which are not fat-soluble also produce more damage following a high-fat diet. Therefore other explanations, such as mechanically impaired circulation [1497] or functional inefficiency owing to relative paucity of normal cytoplasm in the fatty liver, have to be considered.

High-fat diets increase toxicity as measured by necrosis in chloroform [1213], acute [833, 1047] and chronic [1326] carbon tetrachloride, chronic TNT [1498], and acute phosphorus and arsenic [2271] intoxications.

HIGH-PROTEIN DIET. A high-protein diet in hepatic intoxication provides constituents for regeneration, but it also strains the metabolic capacity of the liver. Consequently, the reported effects of high- or low-protein diets are often contradictory. In the majority of experiments a high-protein diet is beneficial in ameliorating the effects of intoxications. For instance, in acute chloroform intoxication it reduces mortality, protects against necrosis, and promotes repair. A complete protein is better than an incomplete one [1213]. Protection has also been reported in acute arsenical intoxication [2271] and in chronic pyridine [186, 673], butter-yellow [2293], and selenium [833] poisonings. In contrast, aggravation of acute phosphorus intoxication has been observed, and in acute carbon tetrachloride intoxication, some investigators found no effect [1326], while others found an increased tendency for necrosis in animals on high-protein diets [477, 840].

LOW-PROTEIN DIET. Low-protein diets aggravate acute chloroform [833, 1213, 2299], bromobenzene [1821], and chronic TNT [1498] poisoning, while low-protein diets have been found equal to or better than the high-protein diets in acute or subacute carbon tetrachloride intoxication [477, 840, 1326].

METHIONINE ADMINISTRATION. Methionine has been considered the effective principle in the high-protein diet, either because of its lipotropic activity or because it is a source of sulphhydryl groups. It is beneficial even in animals on normal diets with acute bromobenzene [1821] or chronic pyridine intoxication [186, 673], but is ineffective in acute [426, 833, 1517] or chronic [3002] carbon tetrachloride intoxication. After protein depletion it is beneficial in acute but not in chronic chloroform intoxication [833] and beneficial in acute Mapharsen [1221] and chronic butter-yellow [1320] intoxication. Methionine was also shown to facilitate recovery from the cirrhosis of chronic carbon tetrachloride intoxication [3000].

CHOLINE ADMINISTRATION. In addition to its lipotropic effect in low-choline, low-methionine, or low-protein regimens, choline reduces necrosis in acute phosphorus intoxication [258, 261] and aids in the recovery from cirrhosis resulting from chronic carbon tetrachloride [3000] or butter-

yellow [1320] intoxications in the presence of protein depletion. It has no effect in acute chloroform [1213], acute [426, 833] or chronic carbon tetrachloride [3002], and selenium [3002] intoxications.

VITAMIN ADMINISTRATION. A protective effect of vitamin E after protein depletion [1553, 1555] and of vitamin B₁₂ on normal diets [718, 1555] in acute carbon tetrachloride intoxication has been described [1553, 1555]. Yeast affords some protection in chronic carbon tetrachloride intoxication [2659]. Liver extracts are protective in acute carbon tetrachloride and chloroform intoxications [833] and in chronic butter-yellow intoxication [1384].

Interrelation of Beneficial Effects. From many heterogeneous experiments, several facts emerge which apply to all types of intoxication. A high-carbohydrate diet is beneficial, and a high-fat diet is detrimental. Low- or high-protein diets are detrimental in certain circumstances and beneficial in others. A normal or somewhat increased protein diet is preferable. Comparison of high-carbohydrate and high-protein diets has led to controversial results. Some investigators prefer high-protein diets in chloroform [1213, 2299], carbon tetrachloride [1319], and arsenic [2271] intoxications, while others achieved better results with a high-carbohydrate diet in chloroform [2760], carbon tetrachloride [334], and phosphorus intoxications. A normal-fat, high-carbohydrate, and normal-to-slightly-elevated-protein diet seems optimal for any intoxication. Dietary supplements seem to offer disappointingly little. Vitamins are beneficial only under certain circumstances; methionine is useful when it acts as a specific detoxicant or in low-protein diets, where its effect is similar to that of choline.

EFFECTS OF ALCOHOL ON THE LIVER

The liver is an important site of alcohol oxidation. The original concept of a toxic effect of ethyl alcohol is gradually being replaced by the idea that nutritional deficiency is the most important mechanism of the hepatic changes associated with chronic alcoholism [1650]. A toxic effect of alcoholic beverages has been ascribed to the presence of alcohols other than ethyl alcohol. In acute alcoholic intoxication, disturbance of hepatic circulation can be assumed.

Studies in Animals. A toxic effect of alcohol in acute experiments is suggested by reduced dye

clearance [2159] or by reduced cholic acid output [238] simultaneously with rapid appearance of fat in the centers of lobules [894]. Under these circumstances, hepatic circulation is probably impaired, since doses producing semicomatose states are given in some experiments [2159].

In chronic experiments, dietary imbalance is more important than toxicity. After deficient diets were shown to produce cirrhosis [2077], chronic administration of alcohol with apparently adequate diets was also found to result in cirrhosis [112, 640]. Subsequently, the adequacy of the diets in these experiments was questioned [259, 531, 833]. High concentrations of alcohol do not produce fatty liver or fibrosis in animals on normal diets. With diets containing marginal amounts of choline and methionine, fatty liver is produced by alcohol intake or its caloric equivalent in sugar [259, 1788]. This suggests that alcohol produces an imbalance between caloric intake and the supply of accessory food factors, particularly the lipotropes. In addition choline requirements are increased beyond this by alcohol [1790]. Correction of the diet by adequate supplementation of choline or methionine prevents the lesion. This may correct the reduction of the alcohol oxidation by the liver caused by protein depletion, which is similar to the reduction of other oxidative enzymes in the liver. Whatever the mechanism, the continued use of alcohol prevents the removal of excess fat from the liver [2658].

Pathogenesis of Hepatic Alterations Caused by Alcohol. The main structural lesion produced by alcoholism, namely the fatty liver, has been explained by several theories. Some are:

1. A toxic effect of alcohol upon the hepatic cells has been claimed even with adequate food intake [112, 2077].
2. Substances such as copper or phosphorus in alcoholic beverages are toxic [2187].
3. Alcohol aggravates the effect of other undetermined hepatotoxic substances [200], as it does the effects of chloroform or carbon tetrachloride.
4. Drunkenness reduces food intake, resulting in protein or vitamin deficiency [1650, 2531], although alcohol produces fatty changes even on a high-protein diet [531].
5. Alcohol produces a gastroenteritis [1497, 2452] and pancreatic insufficiency, which in turn cause secondary malnutrition [1497, 2452].
6. The increased calories from alcohol produce relative deficiencies of lipotropic substances.
7. Alcohol specifically increases the requirement

for choline [259, 1790], which may explain the occurrence of cirrhosis in alcoholic persons with relatively adequate diets.

No definite evidence exists of a reduction of hepatic blood flow through the liver in alcoholic persons. However, the splanchnic oxygen consumption is increased in alcoholic persons with fatty livers and returns to normal upon treatment [1733]. Therefore, one must assume a centrolobular hypoxia that is aggravated by disturbances of sinusoidal circulation in acute alcoholic bouts, producing a shocklike picture. The major importance of nutritional factors in fatty liver and cirrhosis in alcoholic persons and the similarity of the functional and structural changes in animal experiments and in man justify the inclusion of the lesions found in alcoholic persons under nutritional hepatic injury.

Studies in Man. **FUNCTIONAL CHANGES.** Healthy gainfully employed persons can consume relatively large amounts of alcohol without causing significant abnormalities in the hepatic tests [199, 3707]. In chronic alcoholic patients treated in institutions for alcoholism, abnormal results are found in various tests [431, 517, 1953, 3428]; only 10 per cent of patients were found to have normal results in all tests [3428]. The most frequent abnormalities

were, in the order enumerated, elevated total serum-bilirubin level, Bromsulphalein retention, increased urinary urobilinogen excretion, increased serum-alkaline phosphatase activity, and abnormal cephalin flocculation and reduced hippuric acid synthesis [1953, 2881, 3428]. Alcohol-tolerance tests also show abnormal results [721]. Little correlation is found with the appearance of the liver [2881].

STRUCTURAL ALTERATIONS. Fatty liver and cirrhosis frequently occur in alcoholic persons, and alcoholism is the most common cause of the fatty liver-cirrhosis syndrome in the Temperate Zone (see Fatty Liver-Cirrhosis Syndrome from Malnutrition, Chap. 51). In addition, centrolobular necrosis occurs after excessive alcohol intake but is probably the result of disturbed hepatic circulation owing to shock. Such episodes probably explain the large necrotic areas and collapsed bands sometimes seen in alcoholic cirrhosis. Biopsies in alcoholic patients with delirium tremens have indicated normal liver structure, fatty liver, and cirrhosis, each in about one-third of the patients; these findings were poorly correlated with functional alterations [1936]. The structural abnormalities improved with correction of dietary deficiencies and withdrawal of alcohol.

NUTRITIONAL HEPATIC INJURY: CLINICAL ENTITIES

The concepts derived from studies on experimental animals, such as those concerning the lipotropic-lipogenic balance with the effect of starvation, conditioned deficiency, and metabolic interrelated heterogeneous factors, assist in the clarification as well as classification of hepatic injury in human malnutrition. In man deficiency of a single nutrient rarely exists. Imbalance is probably the most important factor in all types of human malnutrition. Relative deficiency of protein plays an important role because of reduction of intake or defects in the nature of the protein (biologically incomplete plant proteins). Malnutrition may not be associated with imbalance, as in starvation when brown atrophy is produced, or it may be associated with imbalance, which leads to the fatty liver-cirrhosis syndrome, the most important nutritional hepatic injury. In the Temperate Zone this is usually associated with alcoholism. In addition human nutritional hepatic injury is caused by disease, disturbances of food absorption, inadequate diets because of either poor voluntary selection, as in fads, or social and economic reasons chiefly seen in the tropics. The opinion has been expressed that diffuse septal cirrhosis develops in various countries on a nutritional basis without a preceding stage of fatty metamorphosis. This requires confirmation by serial biopsies.

Classification

Fatty liver in obesity

Brown atrophy of the liver from undernutrition

Simple nutritional, or alcoholic, fatty liver

Nutritional fatty liver with hepatocellular degeneration

Nutritional fatty liver with cirrhosis

Transition of fatty liver into cirrhosis

Rapid transition into cirrhosis (florid cirrhosis)

Nutritional, or fatty, cirrhosis

Tropical malnutrition

Fatty Liver in Obesity

Necropsy studies on obese people dying suddenly revealed a high incidence of fatty liver [2699]. In 50 per cent of obese persons, fatty metamorphosis was found in liver biopsy specimens [3700]. This was often associated with degenerative or inflammatory changes, depending on the duration rather than the degree of obesity. Hepatocellular damage associated with the fatty metamorphosis has been considered to result from the increased metabolic strain caused by the high-caloric diet. Increased Bromsulphalein retention was the only abnormal result in the hepatic tests. Glucose tolerance was impaired in half of the cases studied in one group [3700], and the hyperglycemia commonly seen in obese persons has been related to hepatic dysfunction [2432], specifically to fatty metamorphosis [3042]. The incidence of cirrhosis in obesity is higher than in the population at large, according to insurance statistics [3700].

Hepatic Brown Atrophy from Undernutrition

FUNCTIONAL ALTERATIONS. Clinical information as to disturbances of hepatic function in starvation and undernutrition comes largely from three sources: (1) areas of famine; (2) besieged cities, such as Warsaw during World War II; (3) prisoners of war or inmates of concentration camps. In these conditions other factors, such as diseases and stress, are present, and laboratory studies are almost impossible. Even from German concentration camps, despite pseudoscientific endeavors, no usable information has been obtained [1603]. In undernutrition, urinary urobilinogen is increased

and fecal urobilinogen diminished, whereas the blood sugar, glucose tolerance, and hippuric acid synthesis are not regularly altered [3045]. In repatriated prisoners of war, the incidence of abnormal Bromsulphalein retention and cephalin flocculation varied [1793, 2255]. In volunteers calorically undernourished the total serum protein and all of its fractions decreased, as did serum cholesterol. Ketosis and ketonuria, hypoglycemia, and a poor response of the blood sugar to epinephrine were also noted [1736]. The manifestations disappeared following protein supplementation. Gynecomastia appearing temporarily after repatriation in some of the prisoners of war was considered to result from inability of the liver to inactivate estrogens [842, 1793].

STRUCTURAL CHANGES. The basic hepatic alteration in undernutrition is brown atrophy. The liver loses more weight than any other organ in the body [1736], as much as five-sixths of its protoplasm disappearing in some instances [3384]. Histologically, the hepatic cells and their nuclei are small, and the cytoplasm is free of glycogen and usually dense [1736] (Fig. 89A). Iron pigment and slightly fluorescent brown wear and tear pigment are present in the hepatic cells and in the frequently proliferated Kupffer cells [2602]. Fat is absent in uncomplicated starvation [3384]. Fatty metamorphosis and inflammatory changes, if present, probably result from intercurrent infections.

FATTY LIVER-CIRRHOSIS SYNDROME FROM MALNUTRITION

Fatty metamorphosis of the liver frequently accompanies many anoxic or toxic conditions as a clinically insignificant feature which does not last long. Fatty changes resulting from nutritional imbalance persist and become the structural basis of an independent disease entity, the fatty liver with its sequelae of diffuse septal cirrhosis. A gradual transition can be observed from (1) an increase in central and periportal fat to (2) diffuse fatty metamorphosis to (3) formation of fatty cysts to (4) fibrosis and (5) cirrhosis. Different stages of this arbitrarily divided process can be seen in the same liver. In the Tropical Zone, malignant malnutrition, or kwashiorkor, is the chief cause, while in the Temperate Zone chronic alcoholism ranks first, accounting for the terms "alcoholic fatty liver" and "alcoholic cirrhosis" [332, 1649]. Delirium tremens and alcoholic psychosis are often

present. Nutritional disturbances from primary gastrointestinal diseases such as pancreatitis, enteritis, and ulcerative colitis far less frequently initiate the same syndrome. Exceptionally other causes of malnutrition or hormonal disturbances appear to be responsible, especially in children, in whom the liver is more sensitive to malnutrition.

Frequently seen evidences of malnutrition in other organs are glossitis, peripheral neuritis, Wernicke's hemorrhagic polioencephalopathy, acute pancreatic fat necrosis, and pancreatic fibrosis. Nutritional heart disease, as characterized by dilatation of both ventricles and mural thrombi in both apices in the absence of alterations of coronary arteries, is exceptional [301]. However, changes in the serum proteins in cirrhosis are supposed to damage the myocardium [2491].

The Role of Complicating Factors in the Nutritional Fatty Liver-Cirrhosis Syndrome. The pattern of the fatty liver-cirrhosis syndrome is modified by many factors. They account for the great variations in the course of the disease and may be responsible for death from hepatic insufficiency in almost any stage and progression of the syndrome from fatty liver to cirrhosis (Fig. 164). Fatty liver in man does not necessarily lead to cirrhosis [739, 783, 1318], although this has been claimed [640, 1407, 1497]. Apparently factors such as necrosis, regeneration, and inflammation, with subsequent variation of the liver tissue in time and space, seem to initiate the transition of fatty liver into cirrhosis [2630, 2647] (see Morphogenetic Pathways, Chap. 28).

The following factors may be responsible for progression of the syndrome, for catastrophic hepatic failure, and for its varied symptomatology:

1. Intercurrent infections, such as pneumonia, upper respiratory disease, and pulmonary and peritoneal tuberculosis, aggravate the condition [943, 1351, 2106, 2804]. In the tropics, malaria or other parasitic diseases may have a similar effect on kwashiorkor [739, 1172]. Patients with a fatty liver or cirrhosis have a lowered resistance to such infections, partly because of the hypoproteinemia. Moreover, the liver of such patients seems to be more susceptible to the toxic effects of such infections. The presence of the infective agents in the liver does not seem to be required, because bacteria and specific granulomas are usually not found [2648]. The possibility has been entertained that the infections somehow facilitate a hypersensitivity reaction, as reflected in the associated elevation of

serum-gamma globulin level. Viral hepatitis is not a factor in these aggravations of the fatty liver-cirrhosis syndrome even if they are associated with severe jaundice. Anatomically, viral hepatitis is not seen under these circumstances, but the morphologic appearance of viral hepatitis in a fatty liver is not established.

2. Anemia and anoxia modify the syndrome. They frequently result from hemorrhage from esophageal varices, peptic ulcers, or from esophageal and gastric tears caused by retching in alcoholic persons (the Weiss-Mallory syndrome). Disturbance of hepatic circulation, especially if shock is present, is an important feature.

3. Cardiac failure from various conditions aggravates the syndrome [3589].

4. Toxic factors in alcoholic beverages may play a role in alcoholism. They are probably more important in tropical malnutrition, in which the effects of bush tea [379, 1491] and senecio alkaloids [3006] are encountered.

5. Genetic and constitutional factors are of importance but are poorly understood [2719]. Males with female body characteristics (Chvostek habitus) are said to have an increased tendency for cirrhosis formation.

6. Endocrine factors influence the progression of the fatty liver-cirrhosis syndrome.

7. Starvation, caused either by lack of food or by terminal diseases, may reduce the fatty metamorphosis without improving the clinical picture. This, as well as changes in eating habits, explains

the frequent absence of fat in the cirrhotic stages of the fatty liver-cirrhosis syndrome.

Simple Nutritional Fatty Liver (Fatty Liver in Alcoholic Persons)

Any alcoholic person with hepatomegaly is presumed to have a fatty liver. Fatty metamorphosis was found in over 70 per cent of biopsy specimens from alcoholic patients [2993]. Only 14 per cent had no abnormal findings; in the remainder fibrosis and inflammatory changes were present. The type of alcoholism seems to be important. The steady drinker who eats at least one good meal has more tendency to exhibit fibrosis than fatty metamorphosis, while the reverse is true for the intermittent drinker with delirium tremens [538]. Only liver biopsy establishes the diagnosis of fatty liver and excludes cirrhosis or other types of hepatic enlargements.

Clinical Manifestations. The lesion, as established by liver biopsy, is found predominantly in males and has no characteristic subjective manifestations [431, 1075, 2658, 2881, 2993, 3609]. The liver is usually large, smooth, and firm, but no correlation exists between the size of the liver and the degree of fatty vacuolization. Splenomegaly is rarely found during life but is frequently seen after death because of intercurrent infections [2647]. Ascites, edema, and spider nevi occur exceptionally. Esophageal varices are found more often, but they rarely bleed. Jaundice is mild if present. Accompanying vitamin deficiencies ex-

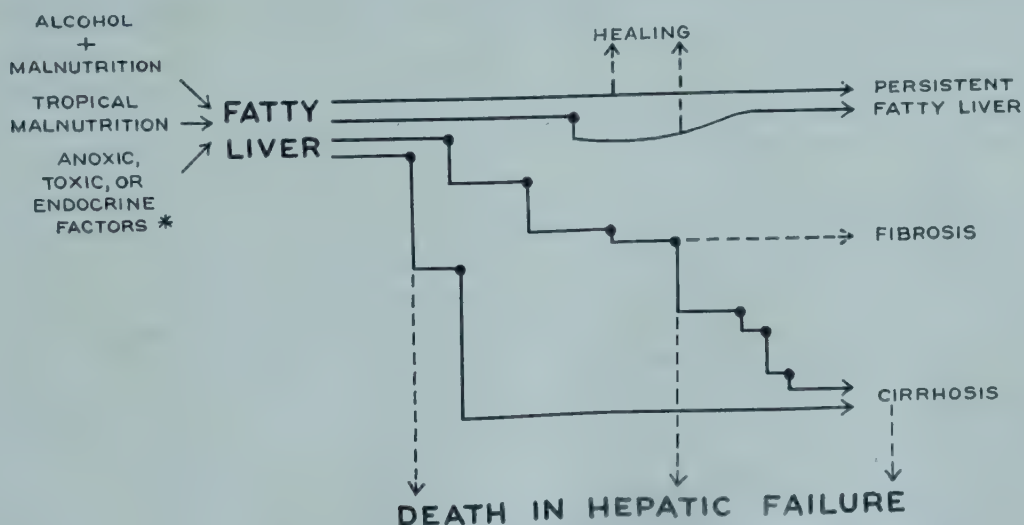


FIG. 164 Course of the fatty liver-cirrhosis syndrome. Black dots indicate episodes of hepatic necrosis, sometimes preceded by infection elsewhere. The factors marked with an asterisk are usually not protracted enough to produce clinically significant fatty liver. (Popper, H., and Schaffner, F.: *A.M.A.Arch.Int.Med.* 94:785, 1954.)

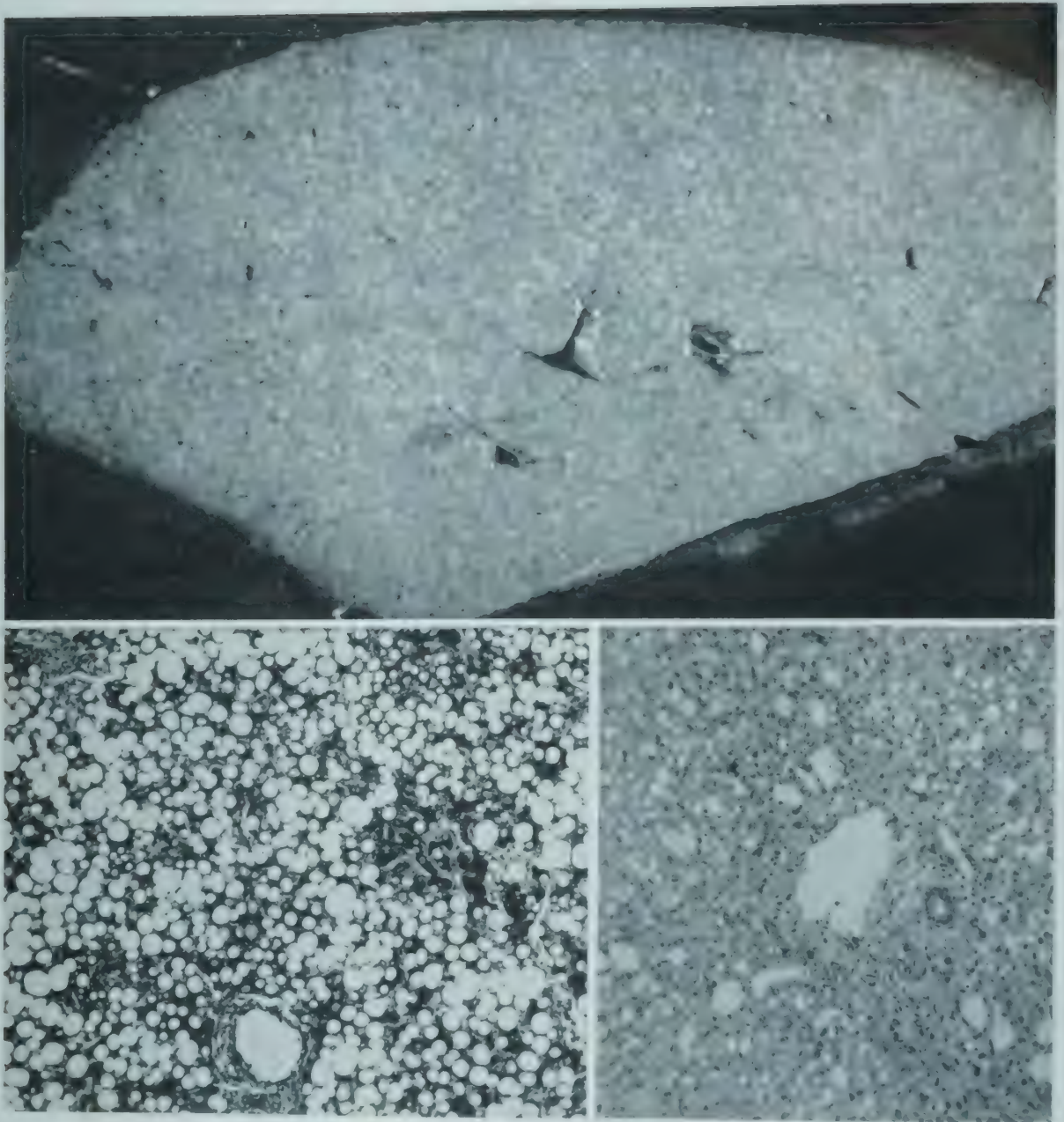


FIG. 165 *Upper.* Diffuse nutritional fatty liver. The architecture is obscured. *Lower left.* Diffuse nutritional fatty liver with little fibrosis. Mallory's aniline blue ($\times 70$). (Popper, H., and Schaffner, F.: *A.M.A.Arch.Int.Med.* 94:785, 1954) *Lower right.* Diffuse fatty metamorphosis with predominantly peripheral accumulation of fat in an alcoholic patient. H&E ($\times 90$).

plain the frequent occurrence of peripheral neuritis and glossitis. The high caloric intake explains the tendency to obesity in spite of the dietary imbalance.

Fatty liver may cause death because of (1) hepatic failure (see Nutritional Fatty Liver with Hepatocellular Degeneration, further on in this chapter); (2) increased susceptibility to infection; (3) sudden derangements not well understood. Respiratory infections are treacherous in patients with alcoholic fatty livers, since fever and other systemic manifestations are often minimal and bring the patients to medical attention late. Sudden death has been reported in this type of fatty

liver and has been associated with metabolic derangements. Fat embolism from fatty cysts has also been considered a cause of sudden death [866].

Laboratory Findings. Survey of over 200 cases reported by various authors [431, 1075, 1938, 2658, 2881, 2993, 3386] indicates that the most frequent abnormal result of the hepatic tests is increased Bromsulphalein retention found in 70 per cent of cases. In about 35 per cent of cases, cephalin flocculation and serum-alkaline phosphatase activity are increased. Serum-gamma globulin level is slightly elevated. Hyperbilirubinemia and reversal of the albumin/globulin ratio occur in 25 per cent of the cases, although in some series

this percentage was higher [1938]. Thymol turbidity is increased in only 20 per cent; reduced cholesterol esters or prolonged prothrombin time are found only exceptionally. Serum-cholesterol level tends to be low. Albuminuria is frequently found. The blood-sugar level is often elevated, but it returns to normal when the fat disappears. The degree of fatty metamorphosis is poorly related to the results of the hepatic tests [2658, 3386].

Structural Alterations. The liver is enlarged and may weigh as much as 5,000 gm (Fig. 165, upper). Its anterior edge is blunted, the capsule is tense, and the color of the smooth surface varies from yellow to greenish brown. The consistency is doughy. On the greasy cut surface, the lobular architecture is obscured. Histologically, the degree of fatty metamorphosis varies from fat deposition in a few scattered cells to diffuse involvement of nearly all cells (Fig. 165, lower left). Usually more fat is found centrally than peripherally, although peripheral fat may be seen without any apparent reason (Fig. 165, lower right). Frequently a "Swiss cheese" appearance is found in paraffin sections from which the fat has been dissolved. The fat droplets are often large and sometimes form fatty cysts. The Kupffer cells are small and fat-free, and the sinusoids are narrow. Beginning perilobular fibrosis gives the portal tracts a stellate appearance. Occasionally septums join neighboring portal tracts. Areas of focal necrosis are sparse, and portal cellular inflammation and ductular proliferation are not conspicuous.

Effects of Therapy. The fatty metamorphosis rapidly yields to dietary therapy and bed rest and usually disappears within 6 weeks [431, 1075, 1734, 1938, 2658]. Its disappearance is associated with weight gain and improvement in the results of the hepatic tests. Intracellular fat is removed much faster than that in fatty cysts [1938]. In contrast, continuation of alcohol abuse or poor dietary intake maintains the fat in the liver. Choline supplements do not improve the effect of a nutritious diet [1734, 2658].

Nutritional Fatty Liver with Hepatocellular Degeneration

In some alcoholic persons with very large fatty livers an acute episode of hepatic failure with jaundice supervenes [1750, 3287]. This may be mild or rapidly fatal. It is usually preceded by severe malnutrition, especially reduced protein intake. A woman working in a candy factory, drinking large amounts of whisky and eating mainly

candy, provided a typical example. The history of such patients often reveals that an additional factor, such as an infection, ushers in the episode of terminal hepatic failure [1938, 2649].

Clinical Manifestations. Clinically, the liver is large and usually tender, but the spleen is not often palpable. Jaundice varies in degree, has a reddish hue, and is sometimes severe. In contrast to conditions in fully developed cirrhosis, the duration of symptoms referable to the gastrointestinal tract is shorter, the patients are younger, and occurrence in the male sex does not predominate [2647]. Edema develops rapidly, especially in fatal cases, and ascites is present in about one-third of cases. Esophageal varices are more common than in simple nutritional fatty liver, and they sometimes bleed. Hemorrhagic tendencies occur in severe cases. Spider nevi and palmar erythema are almost always observed. The mortality varies somewhat, depending on the type of material observed. It seems that about 20 per cent of patients with this condition die, usually in typical hepatic coma. In the remainder, the acute episode subsides within a few weeks. Persisting jaundice and hepatocellular degeneration indicate transition into florid cirrhosis.

Laboratory Findings. Laboratory evidence is found of severe hepatocellular insufficiency and, frequently, of cholestasis, especially if jaundice is present. The cholesterol/ester ratio is very low. Cephalin flocculation and thymol turbidity are often increased, the latter less frequently than the former. Prothrombin time is prolonged, and gamma globulin and the mucoproteins are often increased. The serum-alkaline phosphatase activity may be high, and urobilinogen may be absent from the urine. The differentiation from obstructive jaundice is therefore difficult, at least by hepatic tests [1938, 2649].

Structural Alterations. MACROSCOPIC APPEARANCE. Autopsy studies have been reported under various names, including cirrhosis [1351, 1750]. The liver is large, usually weighing about 3,000 gm but sometimes reaching 5,000 gm. The surface is smooth or only in places finely granular. The consistency is sometimes reduced, despite the doughy character. The yellow or green greasy cut surface shows the lobular architecture to be obscured, except if central necrosis is present, in which case it may be exaggerated. Red bridges may connect neighboring central zones in places.

HISTOLOGIC CHANGES. The architecture is generally intact (Fig. 166A). Fatty metamorphosis is

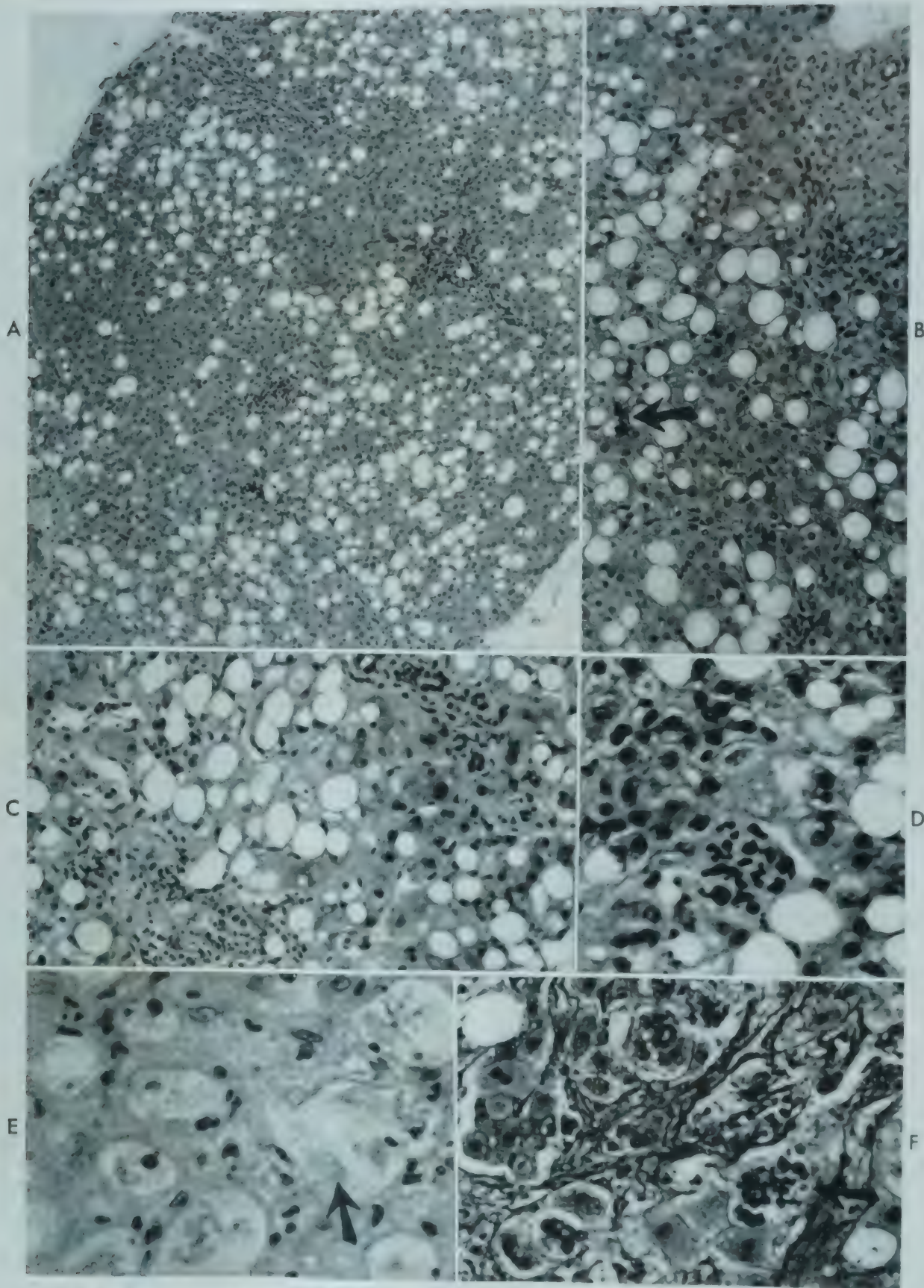


FIG. 166 Biopsy specimens of fatty liver with hepatic failure and jaundice. A. Intact lobular architecture. H&E ($\times 65$). B. Focal hepatic-cell degeneration, bile plugs (arrow), and focal necroses. H&E ($\times 85$). C. Accumulation of neutrophilic leukocytes in streaks around necrotic hepatic cells and around ductules. H&E ($\times 115$). D. Higher magnification showing neutrophilic leukocytes. H&E ($\times 270$). E. Clumps of coagulated cytoplasm in ballooned cells (arrow, "Mallory bodies"). H&E ($\times 270$). (Pepper, H., Sants, P. B., and Parthasarathy, M. *Am.J.Clin.Path.* 25:889, 1955. Courtesy of The Williams & Wilkins Company, Baltimore.) F. Same as in E, stained with Mallory's aniline blue ($\times 220$).

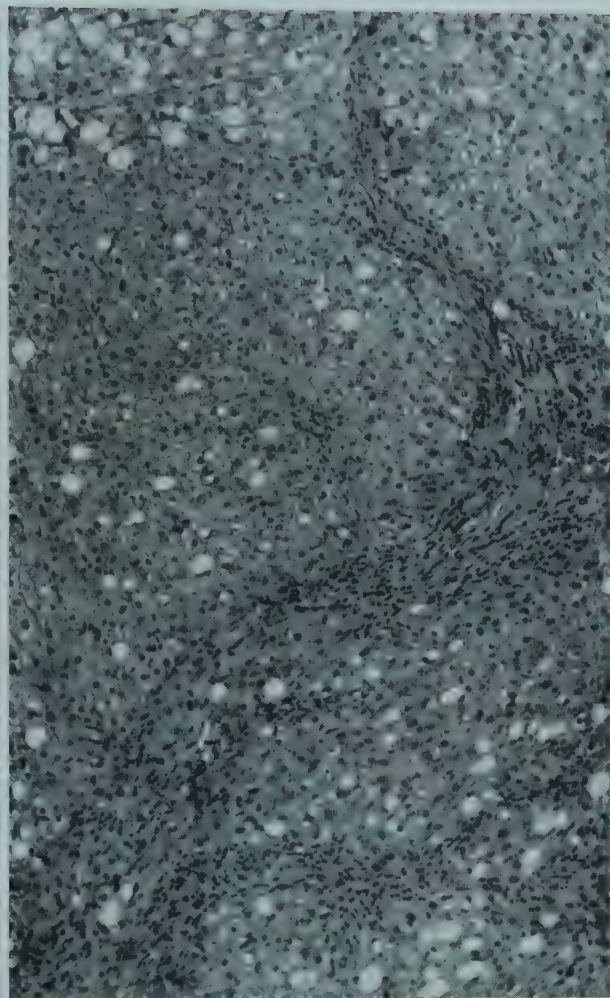
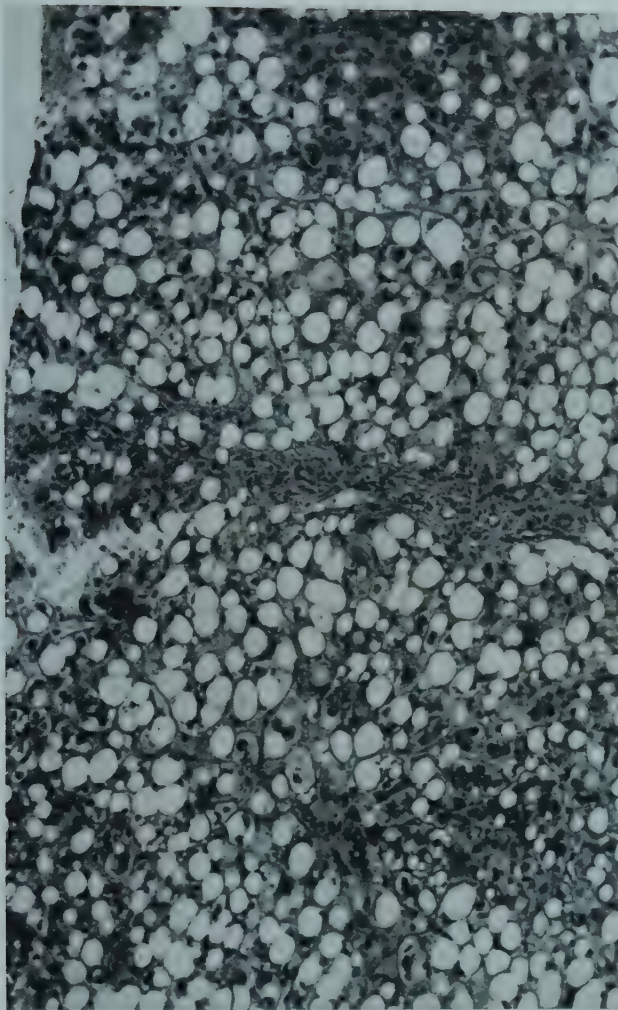


FIG. 167 Biopsy specimens of the same patient taken 2 months apart. H&E ($\times 75$). *Left.* Fatty liver with beginning cirrhosis. *Right.* Fully developed cirrhosis.

usually severe and is frequently accentuated in the centrilobular zone, with the appearance of fatty cysts (Fig. 105B). Sometimes connective membranes radiate from the central fields. The portal tracts are stellate, and collagenous membranes often extend from them into the lobular parenchyma. Perilobular fibrosis is frequently seen; occasionally septums extend into the parenchyma but rarely to the central canal. The lobule is not dissected, and regenerative nodules have not formed, despite active intralobular regeneration, except in a few foci, and therefore cirrhosis is not yet present. The morphologic characteristics suggestive of hepatic failure are not always conspicuous. Hepatocellular degeneration is noted, including circumscribed coagulation of the cytoplasm—the alcoholic hyaline bodies of Mallory [2187] (Fig. 166B, E, F). Focal necrosis is common, while central necrosis is unusual (Fig. 166C, D). Polymorphonuclear leukocytes extend in radiating streaks from the stellate portal tracts, which are infiltrated by in-

flammatory cells, many of which are segmented leukocytes. Bile stasis is usually conspicuous, occurring chiefly in the centers of the lobules. In some instances, not always associated with laboratory evidence of intrahepatic cholestasis, extensive ductular proliferation is seen, with bile plugs within dilated ductules. Fatty metamorphosis is not always diffuse or severe, although the liver remains large. The fat has probably disappeared as a result of changes in nutritional status owing to therapy, discontinuation of alcohol, or starvation, but the hepatocellular degeneration initiated during fatty metamorphosis continues unabated after the partial disappearance of fat.

BIOPSY SPECIMENS. Biopsy specimens reveal the same picture and permit observations on recovery with or without disappearance of fat and on progression into florid or fatty cirrhosis (Fig. 167). Biopsy findings in this condition have been reported in studies primarily concerned with fatty liver or cirrhosis [431, 1938, 2649, 2993, 3441].

The mode of death in these patients implicates hepatic failure. Encephalopathy is usually not found, and pulmonary fat emboli, even if present, do not explain the death. Nevertheless, the correlation between the hepatocellular morphologic changes and the severity of the hepatic failure is poor. A few portohepatic venous anastomoses suggest some shunting of blood from the parenchyma, but this is quantitatively insignificant.

Therapeutic Considerations. Since the acute hepatic failure is frequently ushered in by acute infections, antimicrobial therapy in this stage appears more important than lipotropic therapy. Care must be exercised, however, since some antibiotics may produce untoward hepatic reactions, especially fatty metamorphosis (see Antibiotics, under Idiosyncrasy, Chap. 41). When severe anemia is present, blood transfusions should be given. In the acute stage, when the patients are not eating well, supplemental vitamins and possibly choline or methionine may be useful. A well-balanced, adequate, and appetizing diet is preferred with a low nitrogen content if the patients show any signs of impending coma. In the absence of the signs of frank hepatic failure, protein administration is indicated, in view of the protein deficiency caused by poor food intake, digestive and absorptive difficulties, disturbed intermediary metabolism, and increased excretion of amino acids in the urine.

Transition of Fatty Liver into Cirrhosis

Clinically the transition from fatty liver into cirrhosis may be either inconspicuous and gradual, or dramatic and rapid, the entire process taking place within a few months in florid cirrhosis. Serial biopsy studies demonstrate that the same processes in principle account for both slow and rapid forms. The structural evidence shows that the presence of fat is not essential for progression. Moreover, fat disappearance seems to be associated with increased inflammatory exudation and fibrosis [1075, 1734, 3441] (Fig. 167, left and right). Diffuse septum formation and development of regenerative nodules are the most important pathways, although massive or sub-massive collapse occurs and complicates the morphologic findings. Septums develop (1) around collapsing fatty cysts in the lobular center; (2) from periportal inflammation; (3) because of intralobular necrosis with collapse; (4) in stress fissures which develop because of uneven expansion of hepatic territories owing to necrosis, fatty

metamorphosis, or regeneration. Of these four processes, only the first results from persistence of fat. This explains the variable duration of fatty liver before the development of cirrhosis; it also explains why removal of the fat from the liver by dietary therapy appears to be less important than avoidance of infections, anemia, and acute nutritional deficiencies.

Rapid Transition of Fatty Liver into Cirrhosis (Florid Cirrhosis, Chronic Toxic Hepatitis)

This hepatic disease encountered in alcoholic patients is not easily classified. It has been called "chronic toxic hepatitis" [2632], "subacute portal cirrhosis" [1351], and "progressive alcoholic cirrhosis." The lesion represents subacute or chronic hepatocellular degeneration in a nutritional fatty liver with extensive membrane formation. This, together with conspicuous regeneration, results in a rapidly progressing transition into cirrhosis, although the extent of cirrhosis formation is still minimal. The term "florid cirrhosis" seems to be appropriate [2648]. The hepatocellular degeneration is probably ushered in by intercurrent non-specific infections. Sometimes the fat content is not increased, because of either therapy or, more commonly, starvation, obscuring the connection of this entity with the fatty liver-cirrhosis syndrome.

Clinical and Laboratory Findings. Florid cirrhosis is found slightly more often in males than in females. Most patients concede chronic alcohol abuse and quite frequently show evidence of malnutrition as a result of prolonged starvation or insufficient protein intake.

FATAL FLORID CIRRHOSIS. The duration of symptoms varies from several weeks to many months. They are mostly attributable to the gastrointestinal tract. Weakness and weight loss are present, and upper respiratory infections are common. In the terminal period, gastrointestinal hemorrhage, hemorrhagic tendencies in general, and central nervous manifestations occur. Jaundice is almost always a prominent finding. Ascites develops in half of the patients, and peripheral edema is also frequent. Splenomegaly and spider nevi are found in many instances. The serum protein and serum albumin are usually decreased. Globulins, especially gamma globulin, are frequently increased. The results of the cephalin-flocculation test and the thymol turbidity test are almost always abnormal. The total serum cholesterol is frequently low; serum alkaline phosphatase activity is usually slightly

increased, and in about one-fifth of the cases it is greatly increased. Azotemia is frequent.

NONFATAL FLORID CIRRHOSIS. The clinical and laboratory findings in patients in whom florid cirrhosis is diagnosed by liver biopsy are similar to but less severe than those in fatal cases. Jaundice and laboratory evidence of hepatocellular degeneration are less prominent, but transient periods of cholestasis with severe jaundice and high alkaline phosphatase activity lasting as long as 1 month may occur.

Structural Changes. MACROSCOPIC APPEARANCE. The liver is moderately enlarged, firm, and generally smooth, and its color varies from brown to yellow to green. The doughy consistency of a fatty liver is noted only in some instances. The cut surface presents a polymorphous appearance in some places, while in others it is exaggerated because of enlargement of sunken central zones, which sometimes are connected by bridges of necrosis, resulting in reversal of the lobular architecture. The portal tracts appear enlarged in some areas, and sometimes gray-white connective tissue strands are seen (Fig. 168A).

HISTOLOGIC CHANGES. The polymorphous appearance of the gross specimen is also reflected in the variations of the histologic picture in the same liver and in different livers. Many hepatic cells are severely damaged and frequently show lack of nuclear staining, ballooning, and formation of coagulated clumps, with or without associated bile imbibition (Fig. 168B). Ramified bodies consisting of coagulated cytoplasm (Mallory bodies) are commonly found. They are associated with focal necrosis or inflammatory reactions in which segmented leukocytes participate. The inflammatory exudate accumulates near Mallory bodies but is sometimes seen through the lobule. Streaks of segmented leukocytes extend from the infiltrated portal tracts. Fatty metamorphosis is spotty. The arrangement of the hepatic-cell plates is irregular and often disrupted. Focal areas of regeneration, with hepatic-cell plates several cells thick and binucleated cells, are frequent. Nodules are rare. In addition, central necrosis and submassive necrosis are found in those who succumb (Fig. 168D). Kupffer cell reaction is extensive, and phagocytosis is prominent. Bile plugs are found in the bile canaliculi, and the ductules are often increased and contain inspissated bile. The portal tracts are usually stellate and contain inflammatory exudate, which merges with periportal exudate around areas of periportal necrosis. The character

of the lesion becomes apparent in connective tissue stains, especially Mallory aniline blue stain. Many fine, wavy connective tissue membranes can be noted extending throughout the parenchyma (Fig. 168C). They start from the center or the periphery of the lobules, or they develop independently, usually around areas of necrosis. In some areas, the membranes condense to form septums, producing perilobular fibrosis and exceptionally portohepatic connections containing venous anastomoses. In biopsy specimens the lesion is similar but less severe (Fig. 168E).

CHANGES IN OTHER ORGANS. The spleen is moderately enlarged and exhibits reactive inflammation. Esophageal varices are demonstrable in one-third of cases. The kidneys show icteric nephrosis, with or without acute nephrosis in fatal cases. Acute or relapsing pancreatitis is frequent, and evidence of associated respiratory infections, such as pneumonia or tuberculosis, is fairly common.

Differential Diagnosis. The polymorphous picture often presents differential diagnostic problems [2648]. Grossly, florid cirrhosis differs from fatty liver, with or without hepatic failure, by (1) the polymorphism seen on the cut surface, especially the focal reversal of the lobular architecture; (2) a far less impressive yellow hue and doughy consistency. Fatty cirrhosis shows more extensive nodularity on surface and cut surface (see Structural Alterations, under Nutritional, or Fatty, Cirrhosis, later in this chapter). The size of the liver is no reliable differential criterion, except that it is never so large as in some instances of fatty liver or fatty cirrhosis; i.e., the weight rarely exceeds 2,500 gm. Differentiation from subacute hepatic congestion may be impossible grossly if extreme polymorphism of the cut surface is present in congestion. Histologically, the differentiation from congestion is usually easy, because the membrane and septum formation is not diffuse in congestion but starts from the center. Also, Mallory bodies are not present. Some instances of florid cirrhosis are associated with severe passive congestion, which contributes to the hepatocellular degeneration.

The differentiation from other types of hepatitis is difficult, especially in biopsy specimens. The polymorphism in routine and connective tissue stains is the most reliable differential diagnostic criterion for florid cirrhosis. In florid cirrhosis, in contrast to viral hepatitis, (1) spotty fatty infiltration is noted, while in active viral hepatitis this is present only after antibiotic or cortisone treatment or in children; (2) Mallory bodies, rather than

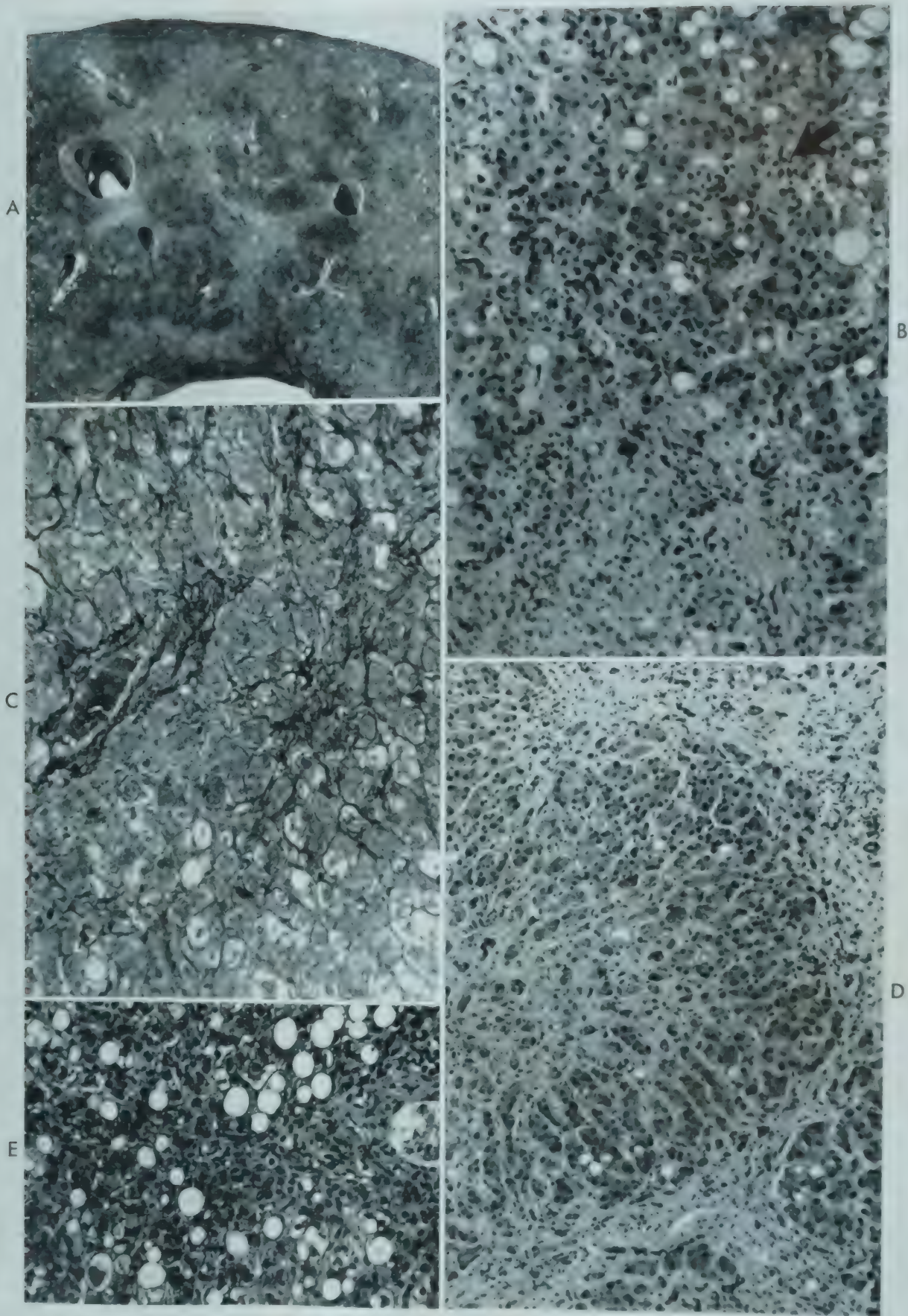


FIG. 168 Florid cirrhosis. A. Smooth cut surface with obscured lobular architecture. B. Diffuse hepatitis, patchy fatty metamorphosis, focal necrosis (arrow). H&E ($\times 130$). C. Diffuse membrane formation throughout the lobule. Mallory's aniline blue ($\times 125$). D. Centrilobular necrosis and collapse with perilobular fibrosis. H&E ($\times 80$). (Figs. A-D from Popper, H., Stante, F. B. and Parthasarathy, M. *Am J Clin Path* 25:880, 1955. Courtesy of The Williams & Wilkins Company, Baltimore.) E. Biopsy specimen showing diffuse hepatitis in a liver with moderate fatty metamorphosis. H&E ($\times 115$).

the diffuse, homogeneous acidophilic bodies; are found; (3) the intralobular and periportal inflammatory exudates contain many segmented leukocytes, while in viral hepatitis mainly mononuclear cells are found; (4) membranes develop irregularly in florid cirrhosis, while their formation is limited almost exclusively to the periportal region in subacute viral hepatitis.

Florid cirrhosis with severe cholestasis must be differentiated from extrahepatic cholestasis with bacterial infection (infected biliary hepatitis). In florid cirrhosis (1) bile lakes and feathery degeneration are absent; (2) hepatic-cell damage is irregular throughout the lobule and usually severe; while in infected biliary hepatitis, centrilobular damage and periportal necrosis stand out against a fairly well-preserved intermediate zone; (3) portal inflammation is usually less severe in comparison with the severity of the intralobular changes, while the reverse is true in infected biliary hepatitis; (4) Mallory bodies, fatty infiltration, and diffuse membrane formation are present, all of which are absent in infected biliary hepatitis.

Toxic hepatitis from chemicals, such as carbon tetrachloride, usually also associated with some fatty metamorphosis, shows distinct zonal, especially central, distribution of hepatic-cell damage, in contrast to the diffuse change in florid cirrhosis. In chemical intoxication, the lesion is spotty in early stages. Central necrosis with collapse is sometimes present in florid cirrhosis, but the diffuse membrane formation present should permit the differentiation.

Nutritional Cirrhosis with Fatty Metamorphosis

Of the various manifestations of the fatty liver-cirrhosis syndrome, the fully developed stage of nutritional cirrhosis is the one which most frequently requires medical attention and therefore is the best-studied stage. In nutritional cirrhosis, the liver passes through stages from a large, extremely fatty organ to a fat-free atrophic one. The fat may disappear in any stage, because of either dietary improvement or undernutrition.

Nomenclature. The presence of fat is not a requirement for the diagnosis, and fat can appear, as a result of malnutrition, in a liver which is cirrhotic from other causes [1172]. These factors obscure the identification of nutritional cirrhosis, especially since the etiologic history is frequently unreliable. Therefore, in the vast amount of literature on cirrhosis, nutritional cirrhosis in the Temperate Zone

is not always clearly separated from other types of cirrhosis, although pathologists have recognized alcoholic cirrhosis [135, 291, 2187], or fatty cirrhosis [1696]. Many instances are seen in a fat-free stage in which the etiology can no longer be proved. Most of the clinical and laboratory information concerns this final stage. Reference is made to the discussion of Laennec's cirrhosis, where criteria of progression also applicable to fatty cirrhosis are discussed (see Rate of Progression of the Cirrhotic Process, Chap. 52). This chapter is concerned with fatty cirrhosis, the clinical and laboratory manifestations of which differ little from those of Laennec's cirrhosis.

Clinical Manifestations. Alcohol abuse is the most important etiologic factor in this country. The terms "alcoholic cirrhosis" or "fatty cirrhosis" have been recommended [135, 291, 332, 1649, 1696, 2187], although alcoholism is only one special form of malnutrition. A male sex predominance of 2:1 is found [943, 1351, 2106, 2719, 2804], associated with the higher incidence of alcoholism in males. The disease is found in all adult age groups, but the majority of patients have the initial symptoms between forty and sixty years of age. The disease occurs earlier in women than in men and predominantly in white persons in the Temperate Zone [943, 1351, 2106, 2719, 2804]. The occupational incidence appears to be based on the incidence of alcoholism [2719].

The most frequently encountered symptoms are abdominal distress and distention, anorexia, and weight loss.

Hepatic failure, often preceded by infections or gastrointestinal hemorrhage, may develop at any time, with its characteristic symptoms. Hepatic failure and acute hemorrhage from esophageal varices are the most common causes of death. The liver is very large and firm, the edge is blunted, and the surface is smooth or finely granular. Jaundice and ascites are present in varying degrees. Splenomegaly is common, while abdominal collateral veins are less frequently seen. Spider nevi and palmer erythema are found in progressing cases. Edema and mild, normochromic, macrocytic anemia are also common.

Laboratory Findings. Bromsulphalein retention is increased. Serum albumin, cholinesterase, and total cholesterol are reduced in the majority of patients, and gamma globulin is increased in more than half of them. The results of the remaining hepatic tests depend upon the degree of associated hepatocellular degeneration and cholestasis. When

hepatocellular degeneration is severe, cholesterol esters are low, urobilinogen excretion is increased, and cephalin flocculation, but not necessarily thymol turbidity, is increased. The degree of jaundice does not always reflect the degree of hepatocellular degeneration. Severe cholestasis, with greatly increased serum-alkaline phosphatase activity and elevated bilirubin and cholesterol levels, occurs without severe hepatocellular degeneration. Sometimes hepatic failure with coma develops without severe jaundice or significant evidence of hepatocellular degeneration. This possibly results from diversion of blood from the hepatic parenchyma via intrahepatic and extrahepatic collaterals (see Hepatic Coma, Chap. 23).

Structural Alterations. **MACROSCOPIC APPEARANCE.** The liver is enlarged, and its greasy, yellow surface and cut surface are granular to finely nodular (Fig. 169A). The lobular architecture is partially or completely replaced by nodules from 1.0 to 8.0 mm in diameter, most of them fairly uniform in size. Some of the nodules, especially the larger ones, are lighter than the rest. The intervening connective tissue is usually a fine network, but frequently wide, fibrous bands are noted, up to 1.0 cm in diameter, in which the normal arrangement of the vessels is not discernible. Fine nodules are seen in these scars. In general the consistency is doughy and is reduced only if severe jaundice adds a green hue to the yellow color.

HISTOLOGIC CHANGES. The lobular architecture is partly or completely abolished by the presence of regenerative nodules, without portal tracts or central canals, and by irregularly arranged septums extending through the parenchyma, frequently connecting portal and central canals. The fat content varies throughout the liver and even within the same nodule. In typical cases, the fat imparts a "Swiss-cheese" vacuolization to the nodules (Fig. 169B). The hepatic cells may otherwise appear unaltered and may show deep basophilia of the persisting cytoplasm. In many cases acidophilic degeneration and focal coagulation (Mallory bodies) occur in fat-free or in fat-containing cells. In autopsy specimens, centronodular necrosis is observed. In both autopsy and biopsy specimens, focal necrosis is frequent. Strandlike aggregations of segmented leukocytes extend from the septums into the parenchyma. Regenerative activity varies throughout the lobule. The nuclei of the fatty cells are frequently large and contain large nucleoli. On the periphery of the nodules, the hepatic-cell plates are several cells thick. The Kupffer cells

are usually inconspicuous. In the presence of severe jaundice, hepatic cells and Kupffer cells are imbibed with bile. Thick bile casts are noted. The ductules are increased in number and contain microcalculi. Connective tissue septums surrounding the lobules or nodules contain a variable number of inflammatory cells, with a sprinkling of segmented leukocytes, moderate ductular proliferation, and fat-containing hepatic cells, singly or in small groups (Fig. 169D). The grossly visible wide bands consist of collapsed connective tissue, in which the irregular spacing of the vessels points to secondary collapse of nodular parenchyma (see Morphogenetic Pathways, Chap. 28). In addition small nodules of fat-containing cells are frequently interspersed. As the fat disappears, the connective tissue septums become scarred, and the lobular architecture is completely abolished (Fig. 169C), the liver shrinks. The criteria for hepatic-cell degeneration and for extent and progression of the cirrhotic process discussed under Laennec's cirrhosis (see Functional Therapeutic Classification, Chap. 52) apply equally to the diagnosis of fatty cirrhosis.

Therapeutic Aspects. The removal of fat from the nutritional fatty liver with or without cirrhosis is not difficult and has been demonstrated by liver biopsy [188, 431, 877, 1075, 1172, 1734, 2587, 2658, 3441]. To obtain this improvement, bed rest, alcohol withdrawal, and a basic diet with a protein content of 1 gm per kg body weight are adequate [1794].

The question whether lipotropic agents, particularly choline and methionine, have an added effect, giving them a practical therapeutic role, has been extensively studied. These substances increase phospholipid turnover in the presence of fatty liver [521], but, with few exceptions [188], choline or methionine given as supplements to a normal diet do not significantly accelerate the removal of fat from the liver [877, 1734, 2658].

Since choline administration to experimental animals on protein-deficient diets removes fat from the liver [259, 1821], it has been recommended for patients who are unable to eat [521]. Well-controlled balance studies in man indicate the predominant role of adequate protein in the removal of fat. A purified diet consisting of glucose with minerals and vitamins fails to remove fat, alter dysfunction, or decrease the size of the liver, whereas oral administration of protein [2587] or intravenous administration of amino acids [876] rapidly removes the fat. Choline given with a

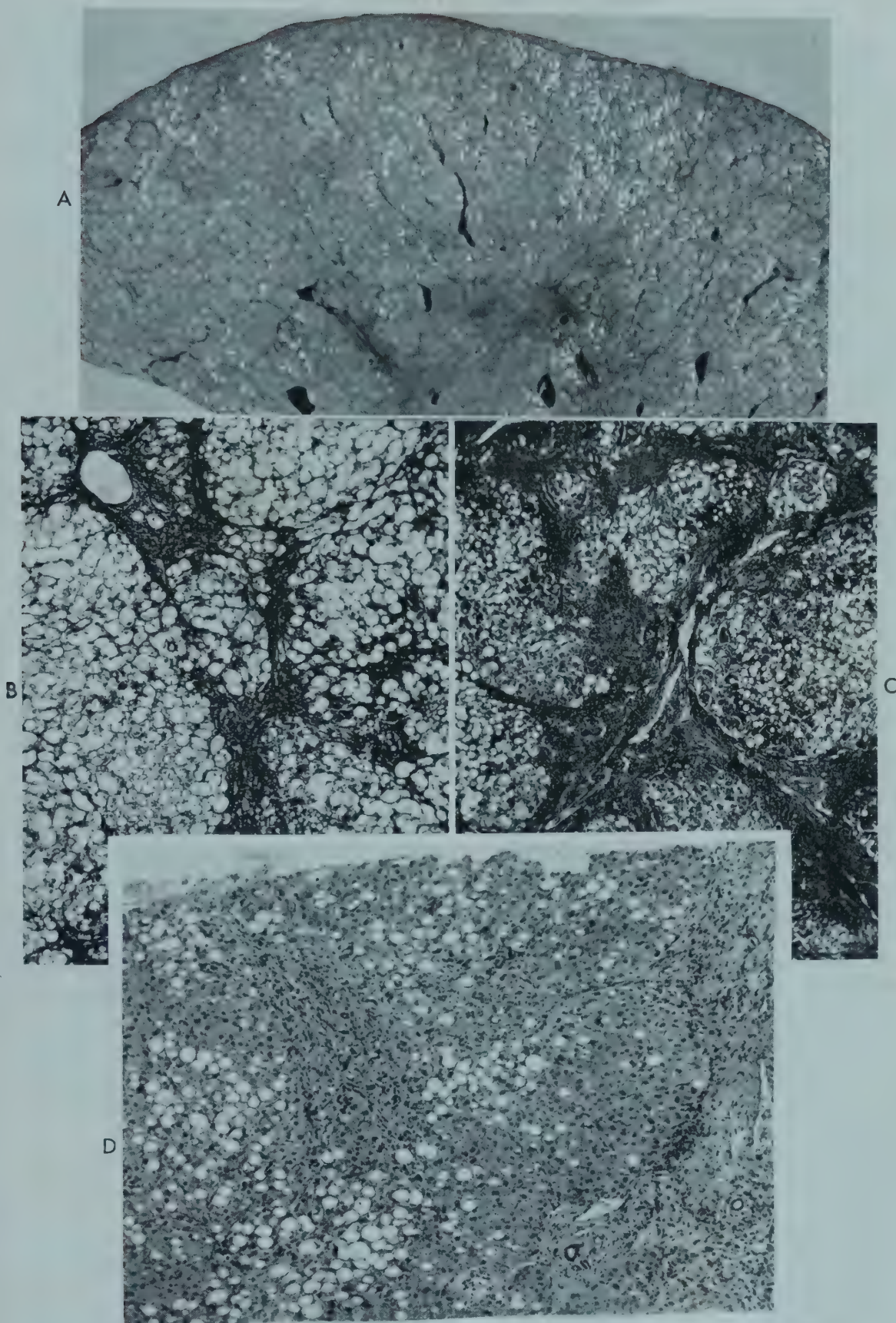


FIG. 169 Fatty cirrhosis. A. Cut surface. B. Beginning distortion of the lobular architecture. Mallory's aniline blue ($\times 60$). C. Complete abolition of lobular architecture and beginning disappearance of fat. Mallory's aniline blue ($\times 60$). (Popper, H., and Schaffner, F.: *A.M.A. Arch.Int.Med.* 94:785, 1954.) D. Biopsy specimen of fatty cirrhosis, showing dissection of lobular architecture by septums of varying thickness. H&E ($\times 60$).

normal caloric but inadequate protein diet reduces fat somewhat [877], but does not alter nitrogen balance in cirrhosis and therefore does not spare protein [1113]. Furthermore, when an adequate diet does not remove fat, choline is also ineffective [1734].

The removal of fat seems to depend upon improvement of the nitrogen balance [877], but impaired hepatocellular function can improve during protein depletion. Fat removal does not arrest fibrosis, nor do supplements have any added effect [1075]. Lipotropic therapy does not significantly prolong the life span of patients with cirrhosis [3449]. Lipotropic supplements are therefore not indicated in the treatment of the nutritional fatty liver and cirrhosis if a nutritious diet is taken [2534].

The therapeutic effects of intravenous administration of concentrated, salt-poor, human serum albumin in this fatty cirrhosis have not been encouraging [972, 3506], although some improvement in liver function has been noted [2344]. A beneficial effect of testosterone is also not established [1113, 1767]. Fat intake in amounts up to 175 gm a day does not seem to be harmful and does not produce fatty infiltration [2531, 3441]. Continuation of alcohol intake in moderate amounts while on a nutritious diet does not prevent fat removal [3441] or clinical improvement [2534]; but continued alcohol intake with a poor diet prevents the disappearance of fat [2658].

Tropical Malnutrition

In all tropical areas of the world, hepatic injury is extremely common, but only in recent years have attempts been made to bring the sketchy information into a unified concept and to clarify the etiologic factors involved. The hepatic injury in the Tropical Zone is a serious geopolitical problem [408]. Lack of personal observation prevents the authors from fully discussing this subject. Instead, the conditions are listed with their potential applications to Western medicine.

Geographical Considerations. Cirrhosis has been known in almost all of the tropical areas for many years and was customarily considered to be the result of tropical diseases, notably malaria, schistosomiasis, amebiasis, and clonorchiasis. With improvement in sanitation and antiparasitic therapy, the effects of these conditions were separated from the ubiquitous tropical hepatic injury. Simultaneously, in several parts of the world, chiefly Africa and Jamaica, emphasis was placed upon malnutri-

tion as the main cause of this injury [1172, 3361, 3362, 3363, 3499]. The concept that this form of cirrhosis was the result of a vitamin deficiency resulted in the name "tropical pellagra," which has now been replaced by "malignant" or "tropical malnutrition," indicating a form of protein malnutrition [739]. Diseases of this type have been described in Central Africa [739, 3361], South Africa [1172], West Africa [2439], the Philippine Islands and Indonesia [3244], Malaya [3330], Jamaica [1637, 3499], and the Dutch West Indies [1410]. A somewhat similar picture is encountered in pellagrins in the United States (Fig. 170A). In recent years, the African tribal name for affected children, "kwashiorkor," has been applied to the condition. It means "red boy," referring to the hair and skin changes which usually occur in the African Negro; the name is now used to describe tropical malnutrition in all ages all over the world [408].

Etiologic Considerations. Extensive nutritional surveys [408] and therapeutic responses [739, 826, 3361] indicate that the basic disturbance in kwashiorkor is not an absolute deficiency of protein, either in total amount or in quality, but rather a deficiency relative to the amounts of carbohydrate or calories consumed. It is thus considered to be analogous to protein malnutrition in childhood in the Temperate Zone [3418], which results from imbalance between protein and carbohydrate intake. Originally a specific deficiency in an amino acid, such as methionine, was held responsible for kwashiorkor. However, lipotropic factors are without effect. Administration of animal protein, especially in the form of skimmed milk, is the best form of therapy [3361]; animal protein is superior to other proteins [3070]. A uniform course of events was originally suggested in tropical malnutrition, starting with fatty liver, progressing to cirrhosis, and terminating in carcinoma. This uniform progression was observed in many areas in which tropical hepatic injury is found, such as South Africa [240], the Dutch Indies [1410], and China [408]. However, subsequent surveys indicated that the geographical incidences of fatty liver, cirrhosis, and hepatocellular carcinoma did not always coincide, and that many years of almost normal liver function intervene between the fatty liver in childhood and cirrhosis formation in adults. Other factors possibly play a supporting role in the development of cirrhosis. Some such possible factors are viruses, parasitic infestations, alcohol, hepatotoxic substances, such as senecio, or bush tea, iron

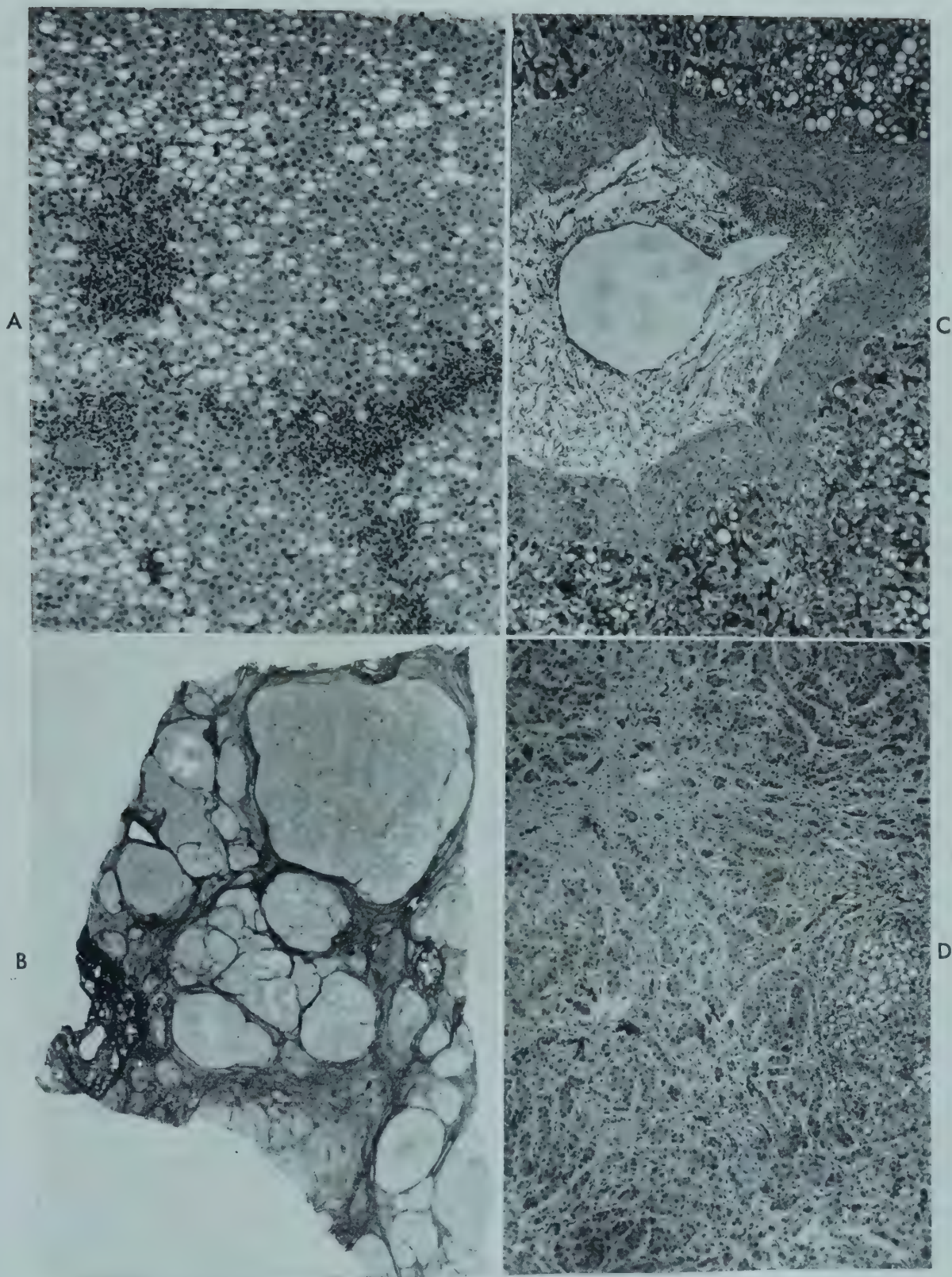


FIG. 170 A. Fatty liver with portal inflammatory infiltration in an alcoholic person with pellagra. H&E ($\times 80$). B. Postnecrotic cirrhosis in an adult African with malnutrition. Mallory's aniline blue ($\times 3$). (Courtesy of Dr. H. Heller.) C. Venocclusive disease in a Jamaican child. H&E ($\times 57$). (Courtesy of Dr. G. Bras.) D. Cirrhosis in a child one year old. The lobular architecture is abolished, and a few nodules contain fat. Extensive regeneration with several-cell-thick plates can be seen. H&E ($\times 57$).

excess, and genetic influences [408]. Until further information is available, it is advantageous to describe a series of entities that eventually may prove to be related to one another.

Juvenile Kwashiorkor. **CLINICAL MANIFESTATIONS.** In African children, in the late breast-feeding and weaning ages, usually at two to three years of age, growth becomes retarded. This retardation is associated with changes in the texture and pigmentation of hair (called dyspigmentation), dermatosis, edema, diarrhea, and anemia [408, 739, 3361]. Mental symptoms, such as apathy and querulous disposition, appear. The children are not cachectic but rather plump, although muscle wasting is evident. At about the age of six, the children recover spontaneously, and their weight curves approach those of European children. The breast milk of the mothers is low in protein and methionine because of deficient protein intake [408, 739], and the children are placed on an adult diet after weaning. This diet contains excess calories but is poor, qualitatively and quantitatively, in protein.

LABORATORY FINDINGS. The total serum-protein level is usually very low [68, 751]. The serum-albumin level is particularly low, whereas the serum-globulin level is normal or even elevated, mainly owing to increased gamma globulin [68], which sometimes rises during treatment [751]. This elevation is not related to infection, but rather to liver disease [408]. The levels of total and esterified serum cholesterol and serum esterase are very low, with a low percentage of esters, while serum-alkaline phosphatase activity is not altered [751]. The thymol turbidity and Takata-Ara tests show abnormal results [3499].

STRUCTURAL ALTERATIONS. The typical morphologic picture was described in Central Africa [739], as well as in Jamaica [1637, 3499] and South Africa [1172]. The liver is moderately enlarged and tawny yellow. Biopsy and autopsy specimens show fatty metamorphosis starting in the peripheral zone and progressing into the centrilobular area. It disappears first from the central zone on treatment. This fatty change is associated with cellular accumulations, especially lymphocytes, in the portal tracts and in the sinusoids. Eventually the reticulum fiber network in the periportal zone becomes thickened and duplicated. Collagen membranes produce a stellate shape of the portal tracts and subsequent perilobular fibrosis. With disappearance of fat, the fibrosis may become aggravated. Exceptionally the fibrosis pro-

ceeds to a fat-free septal cirrhosis with distortion of the lobular pattern. Tissue enzyme activities are low in these livers [1637].

GEOGRAPHICAL VARIATIONS. In parts of Africa, fibrosis without fatty liver has been reported, apparently in children six months of age, but whether this is the same lesion is unknown, since these children do not receive a high-calorie-high carbohydrate diet in contrast to other African children. In the children of some areas such as Central Africa [739], the Fijis, and the Dutch Indies [1410], but not in South Africa or Jamaica, severe atrophy of the excretory parenchyma of the pancreas is seen associated with fibrosis and some interstitial inflammatory infiltration. Ducts or islands of Langerhans are not involved. The pancreatic changes have been assumed to precede the hepatic changes and to contribute to the malnutrition [739] (see Malnutrition, under Parallel Involvement of Pancreas and Liver, Chap. 61). Similar changes were described in parotid glands and the small intestine.

Adult Kwashiorkor. In late adolescence and early adulthood, a form of fatty metamorphosis occurs that is similar to that in juvenile kwashiorkor, although it appears less frequently. The stellate and perilobular fibroses are more severe [739, 1172] and seem to progress to Laennec's cirrhosis sometimes completely fat-free. No sex difference is noted [739]. The hepatic fatty metamorphosis in East Africa is associated with pancreatic changes. Sometimes this cirrhosis seems to develop, even in childhood, without preceding fatty metamorphosis [739]. It is not always of the septal type, and postnecrotic cirrhosis with broad bands is also observed, suggesting preceding massive necrosis and collapse (Fig. 170B). In South Africa the cirrhosis is frequently associated with nutritional siderosis [1172] (see Nutritional Siderosis, under Iron-storage Diseases, Chap. 53), while in East Africa pancreatic fibrosis and endocardial sclerosis have been observed.

Infantile Sclerosis (Venooclusive Disease of the Liver). A special form of hepatic injury has been reported in malnourished children in Jamaica [1491, 1637]. In addition to the typical fatty liver, comparable to that in kwashiorkor [3499], and fatty metamorphosis, associated with hypoglycemia following epidemic vomiting sickness [1491], a peculiar lesion occurs in children, with an apparently good physique, who have an enlarged liver with ascites but no jaundice. Some of these children die in hepatic failure. Levels of serum albu-

min and serum cholinesterase are low. Histologically, chiefly in biopsy specimens [1491], central edema and congestion, or "serous hepatosis," are noted, which progress to new formation of fibers, resulting in centrolobular sclerosis. Eventually perilobular fibrosis develops, which may proceed to cirrhosis. Autopsy specimens of more advanced cases show endophlebitic thickening of the hepatic veins, with eventual complete obstruction, resulting in severe centrolobular congestion and fibrosis [379] (Fig. 170C). The lesion is similar to that in Chiari's disease and also to the changes produced experimentally and clinically by senecio (see Plant Poisons, under Etiologic Factors in Human Toxic Injury, Chap. 41). Senecio alkaloids found in bush tea, a brew made of various plants by the poorer classes of Jamaica, have been assumed to be responsible for the disease. Apparently protein malnutrition predisposes to this lesion, which illustrates the difficulty in separating nutritional from toxic injury [379].

Tropical Juvenile Cirrhosis. In children six to eighteen months of age in various tropical countries, especially India, cirrhosis is relatively frequent. While jaundice occurs usually in the terminal stages, initially the disease is characterized by hepatomegaly and ascites [554]. This condition has been designated as "biliary cirrhosis." The etiology was suggested to be a nutritional deficiency, possibly of choline [554, 2689]. However, in India the disease also occurs in well-fed children, most often boys, thus discounting a nutritional etiology. Other causes, such as bacterial or parasitic infections and toxic or hereditary factors, require further evaluation. Pathologically, the findings have been described by some to be those of a Laennec's cirrhosis [2689]. In some cases the picture is compatible with that of cholangiolitic cirrhosis, in that the lobular architecture is obscured but not destroyed, and relatively few regenerative nodules are seen. Further study is necessary also for clarification of the pathogenesis of the disease.

REVIEW OF THE CLINICAL PROBLEMS AND CLASSIFICATION OF CIRRHOSIS

Cirrhosis as a physiopathologic phenomenon has been discussed already, with no regard to etiology (see Chaps. 28, 29). The main functional manifestations are produced by the formation of regenerative nodules, which produce portal hypertension and abnormal communications between portal and hepatic vessels. These communications are responsible for hepatic-cell damage, shunting of blood away from the hepatic parenchyma through intrahepatic and extrahepatic collaterals, and also for portal hypertension. The hepatic-cell damage and the shunts cause hepatic insufficiency with jaundice, hemorrhagic tendencies, hyperestrogenism, central nervous system manifestations, and eventually hepatic coma. They also contribute to the formation of ascites and edema. Portal hypertension produces the collaterals, especially esophageal varices, as well as splenomegaly with hypersplenism, and contributes to ascites formation. The cirrhotic phase of various diffuse hepatic diseases has also been discussed under the respective disorders. The features of Laennec's cirrhosis and an attempted practical nomenclature require elaboration.

Clinical manifestations usually appear with advanced stages of cirrhosis when the etiologic factors are obscured and the common terminal pathway, Laennec's cirrhosis, has been reached. Many of the clinical observations of cirrhosis thus concern a stage in which the etiology is no longer of importance. This is not necessarily a far-advanced stage, since it includes burned-out initial stages.

LAENNEC'S CIRRHOSIS

The liver is usually smaller than normal, firm, and reddish brown, in the absence of jaundice, or

greenish in its presence. The anterior edge is blunted, and the gallbladder bed is frequently fibrotic. On the surface and cut surface the nodules replacing the lobular architecture are of equal size (Fig. 171, upper left). The intervening firm gray connective tissue produces a regular lacelike pattern. Sometimes the nodules vary in size, and the appearance becomes more polymorphic (Fig. 171, upper right). In typical cases the nodules are of similar size histologically in both fat-free and fat-containing livers (Fig. 171, lower left and right). The intervening trabecular network is dense. Sometimes larger scars, resulting from massive necrosis and subsequent collapse of the cirrhotic parenchyma, are found, especially in nutritional cirrhosis. This secondary collapse is usually located in the part of the liver adjacent to the hilus. Clinical [318, 820, 1030, 2719], laboratory [2662, 2761, 3441, 3467], autopsy [1351, 1400, 1696, 1780, 2719, 2797], and biopsy [96, 744, 2662, 2758, 2764, 3316, 3467] findings of this common terminal stage have been extensively reviewed, and statistical observations are available (Tables 53, 55, 56, and 57).

The incidence of cirrhosis in autopsies varies from 1 to 10 per cent [2719] and reflects social and economic conditions, such as alcoholism and malnutrition. Therefore, charity hospitals have a much higher incidence of cirrhosis than private institutions. The age distribution favors the fifth and sixth decades (Table 53), but cirrhosis in children from various etiologic factors is apparently not so rare as has been assumed [1713] (Fig. 170D), several different types being seen (Table 54). Cirrhosis in childhood is characterized by accentuated regeneration. Children born of cirrhotic mothers are normal, although their serum

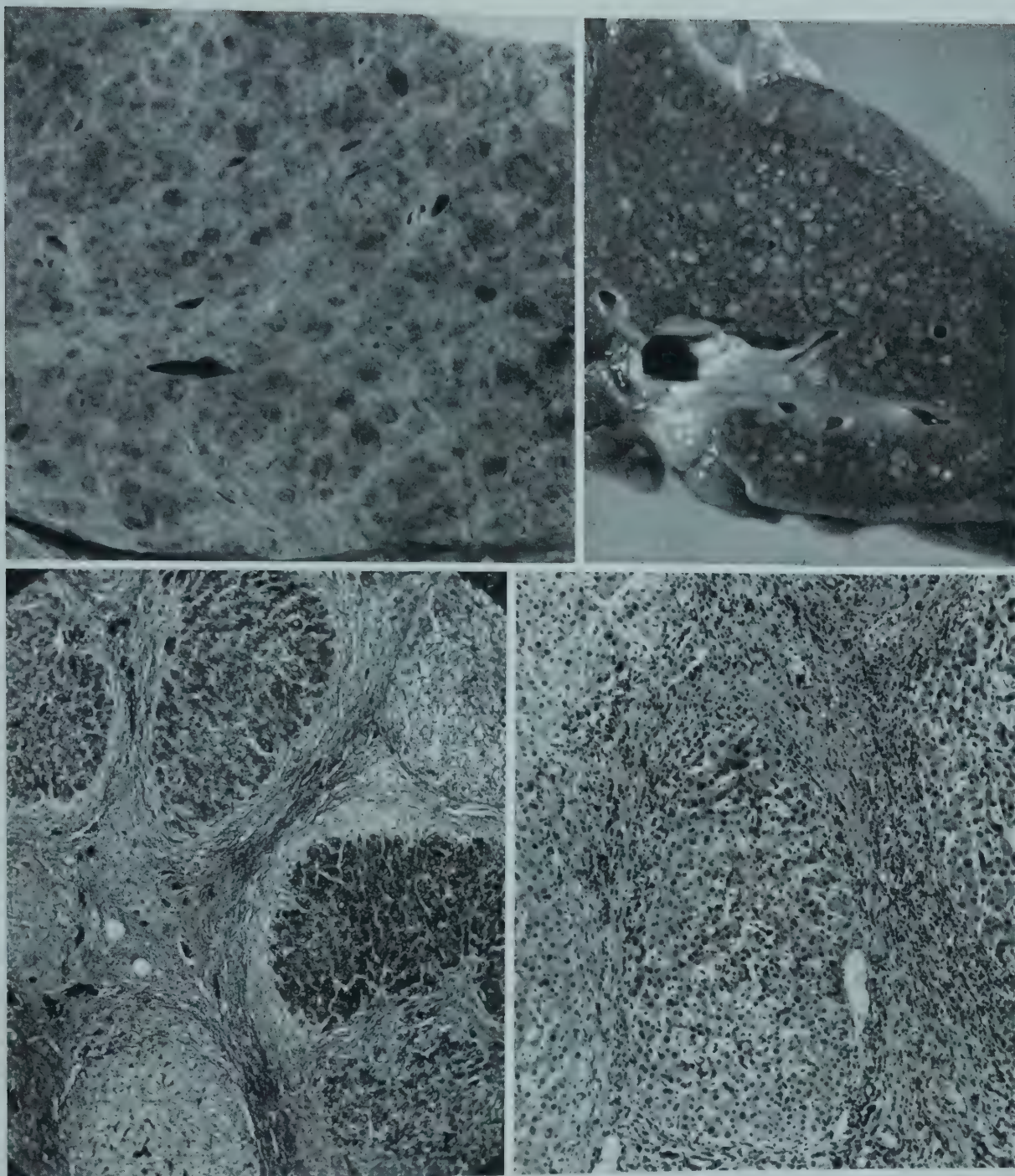


FIG. 171 Laennec's cirrhosis. *Upper left.* Many small nodules (hobnail liver). *Upper right.* Nodules of various size sometimes surrounded by areas of collapse. *Lower left.* Nodules of similar size, some fat-containing, others fat-free, separated by septums of various thickness. Mallory's aniline blue ($\times 60$). (Popper, H., and Schaffner, F.: *A.M.A.Arch.Int.Med.* 94:785, 1954.) *Lower right.* Nodules free of fat. H&E ($\times 55$).

gamma globulin level is usually slightly elevated [3091].

In general, the disease is found more frequently in males (Table 53); the race distribution depends on many factors not well understood. The incidence of cirrhosis of all types in Negroes in this country is significantly lower than in white persons. The incidence of various types of cirrhosis is difficult to evaluate and reflects variations in

clinical material and diagnostic criteria [1696]. In charity hospitals nutritional cirrhosis far outnumbers the other forms, while the reverse may be true in private hospitals and in rural areas. The effect of therapy, changes in the nutritional status of the population, the increasing incidence of viral hepatitis, the amount of alcohol abuse, and racial differences explain varying statistics in different institutions and apparently also in the same insti-

Table 53 Age and Sex Distribution in Cirrhosis

Age	% of sex distribution in 1,198 cases		% of age dis- tribution in 2,508 cases
	M	F	
	% of sex distribution in 2,576 cases		
0-30	2.8	2.9	5.6
30-40	8.3	5.3	14.5
40-50	22.7	10.1	30.2
50-60	23.4	7.7	28.3
60+	12.9	3.8	21.5
	69.8	30.2	

Sources: Armas-Cruz *et al.* [96], Eppinger [943], Fagin and Thompson [965], Fleming and Snell [1030], Hall *et al.* [1351], Henrikson [1462], Kirshbaum and Shure [1780], Ratnoff and Patek [2719], Ricketts *et al.* [2762].

Table 54 Causes and Types of Cirrhosis in Children

Etiology	Morphogenetic type	Page of discus- sion in text
Viral hepatitis (giant cell hepatitis?)	Postnecrotic septal	460
Galactosemia.....	Fatty septal	543
Extrahepatic and intra-hepatic atresia.....	Septal	170
Erythroblastosis fetalis....	Septal	488
Nutritional deficiency.....	Fatty septal	518
Toxicity (bush tea).....	Central septal	519
Congestive failure.....	Central septal	480
Hepatolenticular degener- ation	Fatty septal postnecrotic	540

tution throughout the years. In general the disease seems to have been increasing over the last 30 years.

Clinical Manifestations. The typical patient complains of weight loss, anorexia, fullness in the abdomen, alternating constipation and diarrhea, flatulence, and pain (Tables 55 and 56). Hematemesis and melena are frequent complaints. Unexplained low-grade fever may be present. Jaundice

Table 55 Incidence of Symptoms in Cirrhosis

Symptoms	No. cases reported	%
Weight loss.....	1,521	46
Abdominal distress or distension....	1,450	41
Anorexia.....	1,521	37
Pain.....	1,521	36
Nocturia.....	619	25
Weakness.....	457	24
Nausea or vomiting.....	1,521	22
Hemorrhagic phenomena.....	665	22
Diarrhea.....	1,027	20
Hematemesis.....	1,521	19
Dyspnea.....	619	15
Constipation.....	657	13
Cough.....	486	12
Melena.....	992	11
Pruritus.....	880	8
Oliguria.....	436	4

Sources: Armas-Cruz *et al.* [96], Douglass and Snell [820], Fagin and Thompson [965], Fleming and Snell [1030], Henrikson [1462], Ratnoff and Patek [2719], Ricketts *et al.* [2762].

Table 56 Incidence of Physical Findings in Cirrhosis

Signs	No. cases reported	%
Palpable liver.....	1,979	85
Ascites.....	1,979	85
Jaundice.....	1,979	50
Edema.....	1,521	48
Palpable spleen.....	1,979	40
Unexplained fever.....	1,037	30
Dilated veins.....	1,893	30
Spider angiomas (telangiectasia)....	1,088	26
Hemorrhoids.....	837	19
Scanty body hair.....	758	18
Peripheral neuritis.....	466	12
Hydrothorax.....	664	8

Sources: Armas-Cruz *et al.* [96], Baggenstoss and Stauffer [135], Douglass and Snell [820], Eppinger [943], Fagin and Thompson [965], Fleming and Snell [1030], Henrikson [1462], Ratnoff and Patek [2719], Ricketts *et al.* [2762].

is found in half the cases. Frequently dark pigmentation is noted, caused by increased melanin deposition in the skin [3592]. This has been related to increased levels of estrogens or steroid hormones

in the blood. Ascites is even more frequently found, usually in association with tympanites if the ascites is not too severe. Ankle edema, palmar erythema, gynecomastia, spider nevi, and pectoral alopecia may be present. Axillary hair has usually been lost, and the pubic hair in men has become female in distribution. The fingernails often become white and flat, usually on the thumb first, without changes in the fingertips [3313]. The nail changes are possibly caused by malnutrition [1799]. The parotid glands enlarge, apparently also for the same reason [346]. The liver varies in size from much enlarged to shrunken, depending on the stage of the disease. Nodules can often be palpated, and the edge is blunted. A venous hum sometimes is heard over the xiphoid process and also over the liver directly at laparotomy [2117]; hepatic artery ligation or portal vein occlusion abolishes it. The spleen is usually enlarged, and hydrothorax occurs. Esophageal varices, best detected with the esophagoscope, are often present, but no relationship between their development and other physical findings has been noted [2514]. Hemorrhoids and dilated abdominal veins are also found.

MORTALITY. The causes of death vary in different statistical studies. Hepatic insufficiency usually leads the list, followed closely by bleeding esophageal varices (Table 57). Surgery is poorly tolerated, and postoperative death is frequent.

Table 57 Causes of Death in 535 Cases of Cirrhosis

<i>Causes of death</i>	<i>No. cases</i>	<i>%</i>
Hepatic coma.....	229	43
Hemorrhage (hematemesis).....	163	31
Postoperative death.....	54	16
Pneumonia.....	38	7
Peritonitis.....	23	4
Pulmonary edema.....	7	1
Postparacentesis.....	6	1
Cerebral hemorrhage.....	6	1
Other.....	28	5

Sources: Baggenstoss and Stauffer [135], Douglass and Snell [820], Fagin and Thompson [965], Fleming and Snell [1030], Henrikson [1462], Ratnoff and Patek [2719].

phageal varices (Table 57). Surgery is poorly tolerated, and postoperative death is frequent.

ASSOCIATED DISEASES. Various nonhepatic disorders frequently develop in cirrhosis [2719]. Some

are reflections of protein deficiency, such as increased susceptibility to infection. Tuberculosis, especially tuberculous peritonitis and erysipelas, previously fatal complications [943], has become less common. Lobar pneumonia has a poor prognosis and a high mortality rate in cirrhosis, partly because the symptoms are masked, so that the patient is delayed in seeking medical aid. Portal vein thrombosis occurs and is probably related to portal hypertension. The increased incidence of cholelithiasis is related to disturbances of bile flow (see Causes of Stone Formation, under Gallstone Formation, Chap. 30), while the increased incidence of peptic ulcer and chronic gastritis (see Influence of the Intestinal Tract upon the Liver, Chap. 61) is possibly caused by portal hypertension.

MORPHOGENETIC CLASSIFICATION

A clinical evaluation of the cirrhotic process is based upon a proper description or classification according to morphogenesis, etiology, and functional status.

Three main morphogenetic groups have been recognized: (1) postnecrotic cirrhosis, (2) septal cirrhosis, (3) periductular fibrosis (cholangiolitic or primary biliary cirrhosis) (see Morphogenetic Classification, under Classification of Cirrhosis, Chap. 28).

ETIOLOGIC CLASSIFICATION

The following are accepted as etiologic factors in cirrhosis: malnutrition, including alcoholism, viral hepatitis, intoxications, intrahepatic and extrahepatic cholestasis, granulomatous diseases, parasitic infestations, metabolic disorders, and chronic passive congestion. In many instances the etiology is questionable or can not be ascertained at all. On the other hand, several etiologic factors may be present; for instance, a nutritional cirrhosis may be aggravated by chronic passive congestion, or alcoholism may facilitate the transition of viral hepatitis into cirrhosis.

Recognition of the Etiologic Types of Cirrhosis. Criteria for the recognition of most types of cirrhosis have been presented under the respective disease (Table 58). The main problem is the separation of nutritional cirrhosis from other types. Clinically, this depends mainly on the history; antecedent episodes of jaundice speak against a nutritional etiology [1558].

LABORATORY CRITERIA. In the laboratory, nutritional cirrhosis, in contrast to other types of cirrhosis, is suggested by (1) a relatively low thymol turbidity in the presence of other evidence of hepatocellular degeneration, particularly abnormal cephalin flocculation; (2) moderate elevation of gamma globulin level but not above 3.0 gm per 100 ml; (3) the excretion of coproporphyrin III in the urine; (4) moderate elevation of the serum-mucoprotein level except in the presence of severe hepatic failure, when it is low.

STRUCTURAL CRITERIA. In biopsy specimens, the differential diagnosis of the types of cirrhosis is difficult and often impossible. Usually large pieces of liver are required, such as are obtained at autopsy, and sometimes the entire gross specimen must be studied. Extensive fatty metamorphosis of the lobular and nodular parenchyma suggests nutritional cirrhosis, but fatty metamorphosis on a nutritional basis may complicate other types of cirrhosis. Focal cytoplasmic clumpings, or Mallory bodies, are not found exclusively in nutritional cirrhosis. In general, the following suggest nutritional cirrhosis, rather than posthepatic or post-necrotic cirrhosis: (1) uniform diffuse involvement with thick septums; (2) relatively small and uni-

form regenerative nodules, with complete loss of the lobular pattern in advanced cases; (3) merging of the fibrous connective tissue of the central and portal canals with the membranous connective tissue of the septums. In the postnecrotic or post-hepatic forms, the nodules commonly vary in size and the septums vary in width. Areas of primary massive and submassive collapse are noted, with approximation of normally arranged portal and central canals. The fibrous connective tissue of the central and portal canals is sharply differentiated from the collapsed framework, and, most characteristic of all, the lobular architecture is preserved and normal portal tracts are seen in some nodules. Regeneration is often bizarre, and large multinucleated cells are found. Uniformly thick septums with fingerlike extensions are seen in cholangitic cirrhosis and in hemochromatosis, as well as in some instances of cirrhosis associated with parasitic infestations.

FUNCTIONAL-THERAPEUTIC CLASSIFICATION

Since the etiology of cirrhosis frequently can not be established, the status of a patient suffering

Table 58 Relation between Etiology and Morphology in Cirrhosis

<i>Etiology</i>	<i>Precirrhotic changes</i>		<i>Initial type of cirrhosis</i>	<i>Duration of pure form of cirrhosis</i>
	<i>Type</i>	<i>Duration</i>		
Nutritional imbalance	Fatty liver, necrosis, stress fissures	Few months to many years	Septal	Varies, may be years
Viral hepatitis	Massive necrosis	Months	Postnecrotic	Months?
	Massive or submassive necrosis	Few months	Postnecrotic	Months
Congestion	Spotty necrosis, portal membrane formation	Years?	Septal?	?
	Central collapse and membrane formation	Years	Septal	Years
Toxic injury	Central collapse and membrane formation	Months	Septal	?
Infected biliary hepatitis	Peripheral membrane formation and collapse	Many months to years	Septal	Many months
Hemochromatosis	Peripheral membrane formation	Many years	Septal	Years
Zooparasitic diseases	Peripheral membrane formation and necrosis	Many years	Septal	Years
Granulomatous diseases	Peripheral membrane formation	Many years	Septal?	Years
Biliary hepatitis (noninfected)	Pericholangiolitis	Years	Periductular fibrosis	Years
Cholangiolitis	Pericholangiolitis	Years	Periductular fibrosis	Years

from this disease, which is known for its variety of manifestations, requires description by criteria other than etiologic ones. Three features appear to provide the best basis for an attempt to develop a functional-therapeutic classification such as is found helpful in cardiac and tuberculosis diagnosis. These are (1) the extent to which the cirrhosis has advanced; (2) the speed with which it seems to be progressing at the time of examination; (3) the degree of hepatocellular damage. Hepatic-cell damage is important because, first, it may start the cirrhosis, and, secondly, it is produced by cirrhosis, in view of the alteration of the circulation by the regenerative nodule and by the bypass of the parenchyma by the vascular anastomoses. Such a functional-therapeutic correlation is based on morphologic criteria recognized by liver biopsy. The structural changes do not necessarily show a perfect correlation with clinical and laboratory findings, suggesting that if confusing clinical and laboratory findings are present, liver biopsy may offer the best answer. Selection of the clinical and laboratory criteria given below is based upon clinical-pathological correlation of a large number of cases of cirrhosis studied by liver biopsy [2905] (Table 59).

Degree of Hepatocellular Damage

The presence or absence of hepatocellular degeneration and necrosis is reflected in the degree of hepatic failure. This has also been expressed by the terms "compensated" and "decompensated" cirrhosis [943]. The causes of hepatic failure may be episodes of severe malnutrition, including acute alcoholism, intercurrent infections, hemorrhage from esophageal varices, and factors inherent in or causing the cirrhotic process. Hepatic-cell damage is the aspect which has received much attention in the treatment of cirrhosis, sometimes with gratifying results.

Clinical Criteria. The presence of hepatic failure in cirrhosis is clinically reflected by jaundice, although severe failure can occur without it, and by a tendency for gastrointestinal bleeding. In addition, anorexia, tenderness of the liver, and, in severe cases, fetor hepaticus and hepatic coma are observed. Splenomegaly, edema, spider nevi, and palmar erythema occur in severe hepatocellular degeneration but are not an indication of it, since they may occur in the absence of severe hepatic-cell damage. The same is true of ascites, which depends on several factors.

Table 59 Criteria for Evaluation of Cirrhosis

Degree of hepatic-cell damage	Extent	Rate of progression
None or mild: Flocculation-test results normal Bromsulphalein retention abnormal, urobilinogen level elevated Little if any focal necrosis No symptoms	Minimal: Liver size normal or slightly enlarged Minimal portal hypertension Incomplete division of parenchyma by septums and few nodules	Inactive (arrested): Normal gamma globulin level Minimal inflammation and membrane formation Portal-tract borders sharp Regenerative activity minimal
Moderate: Flocculation-test results mostly abnormal Albumin level low Cholesterol-ester level low Slight to moderate jaundice Extensive focal necrosis and usually diffuse hepatic-cell damage	Moderately advanced: Portal hypertension with splenomegaly Liver size normal or slightly enlarged Lobules subdivided by septums with multiple nodules	Progressing: Elevation of gamma globulin level Severe inflammation and membrane formation Portal-tract borders indistinct Active nodular regeneration Most hepatic test results abnormal
Hepatic failure: Flocculation-test results abnormal Very low albumin and cholesterol-ester levels Jaundice may be severe Ascites Hemorrhagic tendencies Hepatic coma Diffuse hepatic-cell damage with extensive focal and sometimes central necrosis	Far advanced (Laennec's cirrhosis): Severe portal hypertension with splenomegaly Liver small Complete loss of lobular architecture with only nodules remaining	Rapidly advancing: Develops in months instead of years Extensive inflammation and membrane formation Very active cytologic and nodular regeneration Cholesterol-ester level low

Laboratory Criteria. Nearly all hepatic tests show a higher incidence of abnormal results in the presence of severe hepatic-cell damage than in its absence. The findings which best indicate hepatic-cell damage are increased cephalin flocculation and thymol turbidity, hypoalbuminemia, and, to a lesser degree, hyperglobulinemia [2651]. Very high gamma globulin levels are found equally with or without severe hepatic-cell damage. Bromsulphalein retention is also of value, but it depends on factors other than liver damage. Urobilinogenuria is not a reliable index, because it is decreased in the presence of hepatic-cell damage by anemia or renal failure, or increased without significant hepatic-cell damage in hemolysis. Hyperglycemia and acidosis occur, but hypoglycemia has also been found (see Carbohydrate Metabolism, under Chemical Changes in Serum and Urine in Hepatic-cell Degeneration, Chap. 23).

Structural Criteria. Grossly, the consistency of the liver is reduced in hepatic failure. Gallbladder bed edema may be the only other gross indication of hepatocellular injury, since the distorted architecture obscures central necrosis. Histologically, the following criteria apply in biopsy specimens: (1) significant variations in the size and staining qualities of neighboring cells (Fig. 172, upper left); (2) loss of the normal basophilia of the cytoplasm with vacuolization, ballooning, or clumping, resulting in formation of ramified acidophilic perinuclear bodies, or Mallory bodies, which are well defined (Figs. 88C, 166E, F); (3) variations in the size and staining qualities of the nuclei, from pyknosis to complete disappearance; (4) necrosis of isolated or small groups of hepatic cells, with aggregation of segmented leukocytes and some mononuclear cells masking the cellular debris (Fig. 166C, D); (5) hepatocellular necrosis in the centers of regenerative nodules (Fig. 172, lower left). In autopsy specimens, centrilobular and centronodular necrosis is generally more conspicuous than in biopsy specimens. The degree of hepatocellular injury varies throughout the liver. The presence or absence of fatty metamorphosis is not a criterion for hepatic-cell degeneration. Fatty metamorphosis largely determines the size of the liver but only to a minor degree its function.

Jaundice in Cirrhosis. The relation of jaundice to hepatic failure in cirrhosis is not always clearly understood. Statistically, a good correlation is found between the presence and degree of jaundice and histologic evidence of hepatic-cell damage [2152, 2651]. Fatal hepatic coma after a long

period of hepatic insufficiency can occur without severe jaundice. In contrast, patients with severe jaundice are seen in whom hepatic-cell damage is not severe, i.e., in cholestatic cirrhosis. Jaundice in cirrhosis is in part caused by hemolysis, as a result of splenomegaly. In addition intrahepatic cholestasis is sometimes severe, as indicated by the absence of urinary urobilinogen, greatly increased serum-alkaline phosphatase activity, and high total cholesterol levels. The original assumption that this cholestasis is a result of intrahepatic scarring has not been confirmed by biopsy studies [2651]. It is probably an alteration of the ductules, similar to that in other diffuse hepatic diseases.

Extent of the Cirrhotic Process

In autopsy material, cirrhosis is an incidental finding in almost 20 per cent of cases [2106, 2804]. In biopsies cirrhosis may also be an unexpected finding. The extent of the cirrhotic process is more easily recognized morphologically than clinically or by laboratory tests. Of the three factors chosen for the evaluation of cirrhosis, the extent of the process probably has the least functional and therapeutic importance, since it is neither treatable nor reversible. It is important for the appreciation of portal hypertension. The extent of the cirrhosis partly determines the end results of operations for portal hypertension.

Clinical Criteria. As cirrhosis progresses from minimal to moderately advanced to far advanced, the incidence of splenomegaly, esophageal varices, abdominal collaterals, and edema increases. In contrast the incidence of jaundice, ascites, gastrointestinal bleeding, spider nevi, and palmar erythema is much less influenced by the extent of the cirrhosis. Altered reconstruction is usually associated with loss of parenchyma, which is only partially replaced by newly formed connective tissue. Therefore, the more extensive the process, the smaller the liver, the firmer its consistency, and the more easily the nodularity is palpated.

Laboratory Criteria. Of the laboratory tests Bromsulphalein retention is most uniformly abnormal and the degree of abnormality somewhat reflects the extent of the cirrhotic process, because of disturbed circulation caused by both hepatic vein compression and portohepatic anastomoses. Most of the other hepatic tests reflect the extent of the process very little. The percentage of abnormal results in tests of cephalin flocculation, serum bilirubin, alkaline phosphatase activity, and

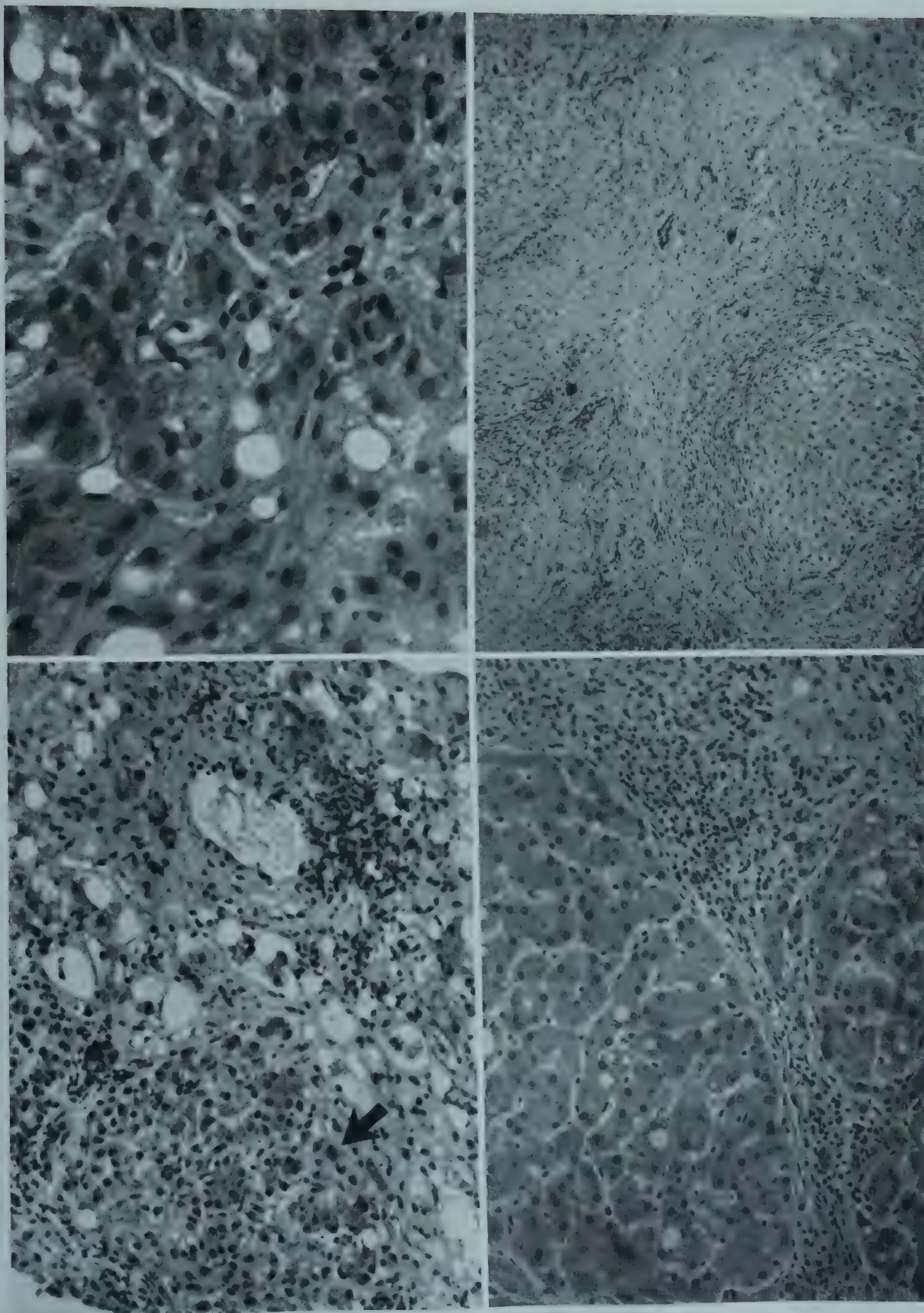


FIG. 172 Features in biopsy specimens of Laennec's cirrhosis. H&E. *Upper left.* Hepatic-cell damage with variations in size and shape of cells and leukocytic reaction ($\times 330$). *Upper right.* Scarring around interlobular bile ducts and periportal ductules, as well as regenerative nodule ($\times 60$). *Lower left.* Portal inflammation and periportal necrosis, ductular proliferation, and hazy limitation (arrow) of regenerative nodule undergoing necrosis ($\times 120$). *Lower right.* Sharp limitation of regenerative nodule with reestablished limiting plate ($\times 120$).

gamma globulin rises slightly as the extent increases. Thymol turbidity and the cholesterol ratio are not influenced. Serum-albumin level has a tendency to be low in far-advanced cirrhosis [3467], but numerous examples of this condition have been recorded in which no hepatic test suggests the disease. It is then known as "latent cirrhosis" [2760].

Structural Criteria. The extent of the cirrhotic process is determined by how much of the original lobular parenchyma is replaced by regenerative nodules (Fig. 174, lower) and the number of septal connections between portal and central fields carrying vascular anastomoses (Fig. 173, upper right). In nutritional cirrhosis the extent of one process usually runs parallel to that of the other. In minimal cirrhosis, few connecting septums and regenerative nodules are seen (Fig. 173, upper left and right). In moderately advanced cirrhosis, the number of nodules and connecting septums is greater (Fig. 173, lower left and right). In far-advanced cirrhosis, the lobular architecture is completely abolished, and the lobular parenchyma is replaced by regenerative nodules (Fig. 174, upper and lower). The presence or absence of fat does not reflect the extent of the process, since fat may disappear relatively early from most of the parenchyma, or it may remain widespread in very late stages with extensive reconstruction. The presence of fatty cysts in the septums is a sign of far-advanced cirrhosis. Similarly, primary carcinoma occurs only in late stages.

Rate of Progression of the Cirrhotic Process

The cirrhotic process may progress at any given time very rapidly, slowly, or not at all. This progression can be designated as the activity of the cirrhotic process, but since this connotation often includes hepatocellular degeneration [2651], the use of the term may be confusing. The rate of progression of the cirrhotic process must be differentiated from its extent, in that a very early cirrhosis or even the precirrhotic fatty liver may actively progress while a far-advanced cirrhosis may be arrested or stationary. Appreciation of the significance of the rate of progression, rather than extent, is reflected in the term "florid cirrhosis" (see Rapid Transition of Fatty Liver into Cirrhosis—Florid Cirrhosis, Chronic Toxic Hepatitis, Chap. 51). The rate of progression deserves special therapeutic attention, since progression can be arrested. Since the active process is associated with

characteristic signs and symptoms, it should be arrested if possible, for instance by prompt therapy for malnutrition, anemia, or intercurrent infections.

Clinical Criteria. Progression may be insidious over a prolonged period of time, or it may be more rapid following episodes of acute malnutrition or hepatic failure, hemorrhage from varices, or intercurrent infections. Jaundice, ascites, spider nevi, palmar erythema, and hemorrhagic tendencies are more commonly seen in progressing cirrhosis than in arrested cirrhosis. Bleeding from esophageal varices is also more common in progressing cirrhosis.

Laboratory Criteria. The incidence of abnormal results of tests of cephalin flocculation, thymol turbidity, serum albumin, serum bilirubin, and cholesterol ester ratio is greater in progressing cirrhosis than in arrested cirrhosis. Elevation of the serum-gamma globulin level reflects progression of cirrhosis better than any other finding.

Structural Criteria. The following findings, usually demonstrable in biopsy specimens, indicate the apparent rate at which the cirrhotic transformation takes place: (1) cytologic evidence of active regeneration in the form of large hepatic cells, with large nuclei and nucleoli, found especially on the periphery of the nodules (Fig. 110C, D); (2) increase in the width of the hepatic-cell plates from one cell in the center of the nodule to several cells irregularly arranged on its periphery (Fig. 117C); (3) extension of collagenous membranes throughout the parenchyma, condensing in places to form septums into which sinusoids are included, forming the vascular anastomoses (Figs. 111, 112); (4) indistinct boundaries between lobular and nodular parenchyma on one side and the septums on the other, resulting from destruction of the limiting plate (Fig. 172, lower left); (5) many inflammatory cells in septums and portal tracts, as well as in the parenchyma, around necrotic foci (Fig. 172, lower left). Ductular proliferation, sometimes surrounded by scar tissue, is frequently present (Fig. 172, upper right). Although some of the processes, such as nodular regeneration and membrane formation, are independent, they usually run parallel to the other processes. In autopsy specimens the presence of stress fissures is also an indication of activity. Fibroblastic proliferation is only exceptionally important. The degree of vascularity of the septums interpreted as angiogenesis [2359], may indicate progression, in that it reflects incorporation of

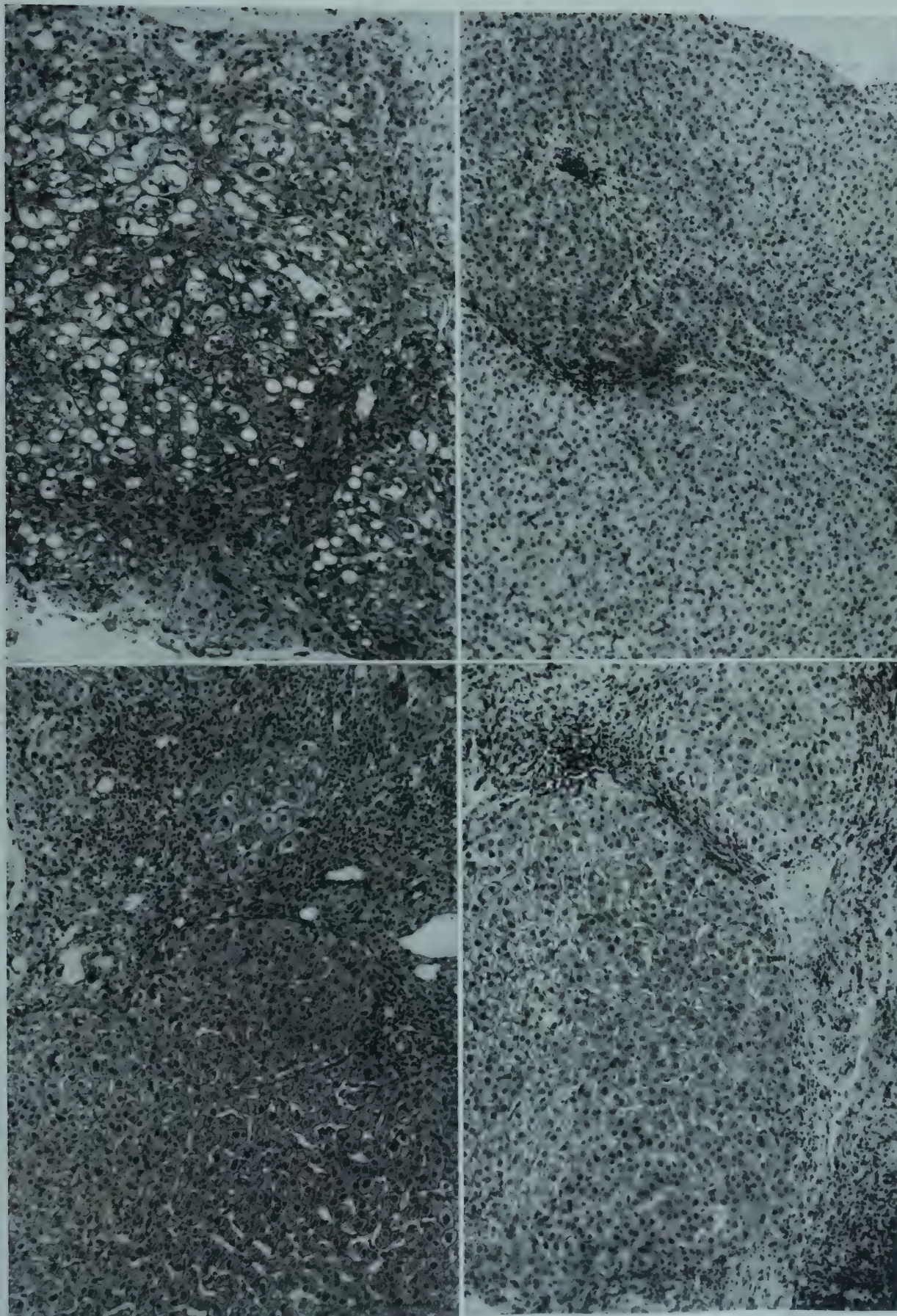


FIG. 173 Stages of cirrhosis as seen in biopsy specimens ($\times 75$). H&E. *Upper left.* Minimal active cirrhosis with marked hepatic-cell damage ($\times 73$). *Upper right.* Minimal arrested cirrhosis with mild hepatic-cell damage and septum connecting central field with portal tract ($\times 75$). *Lower left.* Moderately advanced active cirrhosis with moderate hepatic-cell damage ($\times 74$). *Lower right.* Moderately advanced arrested cirrhosis with moderate hepatic-cell damage. Mallory's aniline blue ($\times 75$).

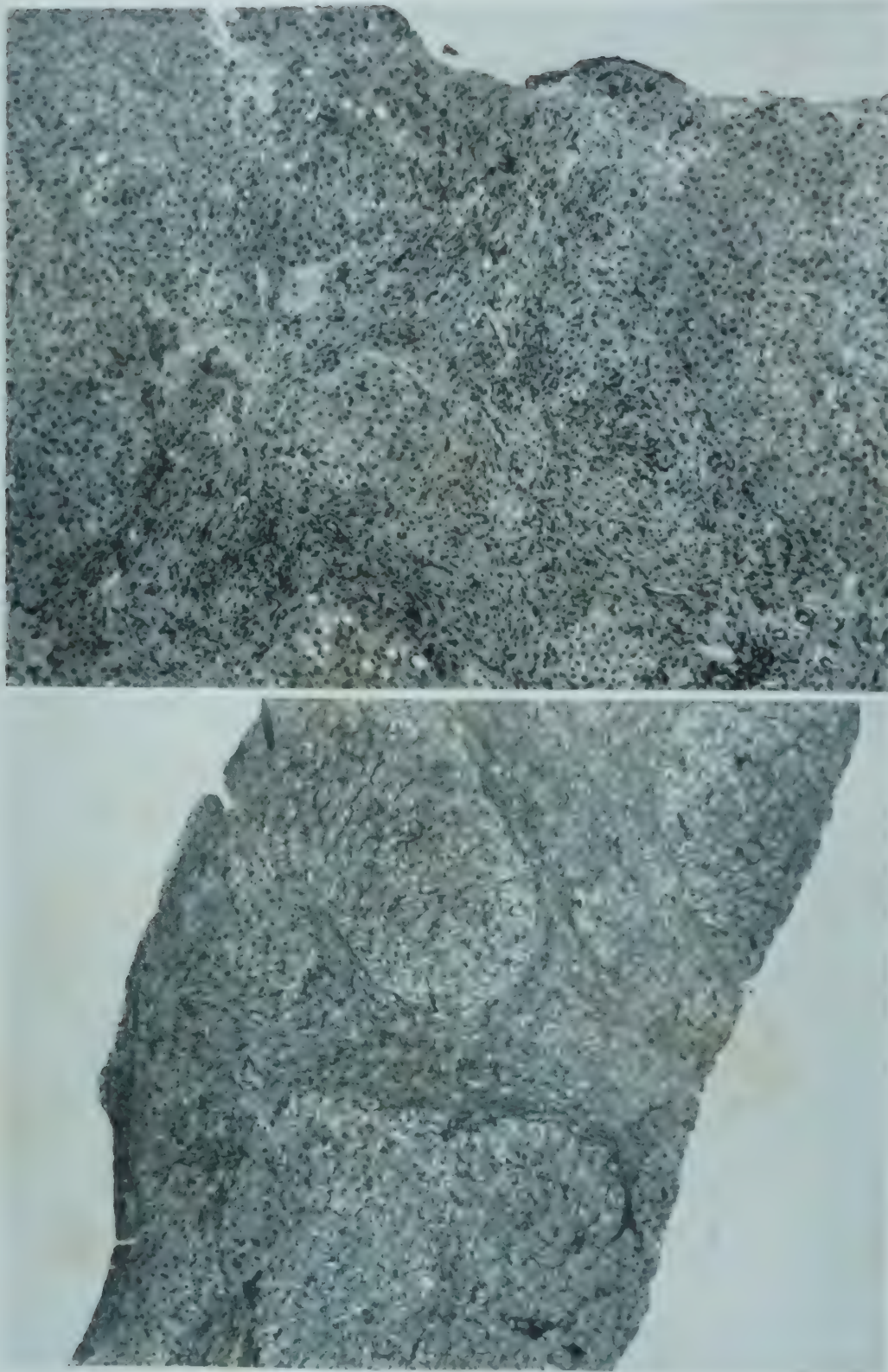


FIG. 174 Stages of cirrhosis as seen in biopsy specimens. *Upper.* Far-advanced active cirrhosis with moderate hepatic-cell damage ($\times 51$). *Lower.* Far-advanced arrested cirrhosis with moderate hepatic-cell damage. H&E ($\times 45$).

sinusoids into the septums, producing portohepatic anastomoses.

Criteria for Arrest of the Cirrhotic Process. The criteria for arrest are (1) lack of signs of active regeneration with radiating arrangement of the hepatic cell plate in the nodule; (2) sharp bound-

aries between parenchyma and septums, indicating reconstruction of the limiting plates (Fig. 172, lower right); (3) few if any inflammatory cells in the parenchyma and in the septums; (4) absence of necrosis. Scarring of the septums does not necessarily indicate subsidence of activity.

PROPOSED NOMENCLATURE

An attempt should be made to describe each case of cirrhosis according to three criteria. A morphogenetic term should be followed by an etiologic one and should be modified by a functional-therapeutic evaluation. The morphogenetic pathway is occasionally obscured, especially in Laennec's cirrhosis. More often the etiology can not be established. Individual cases show unrelated variations in each of the functional-therapeutic categories (Table 59). For instance, one case may be far advanced and arrested and it may show moderate hepatic-cell degeneration (Fig. 174, upper), while another may be early and progressing, with severe hepatic-cell damage (Fig. 173, upper left). The number of possible variations is great (Figs. 173, 174). Moreover, sudden shifts from one group to another occur.

DIFFERENTIAL DIAGNOSIS OF CIRRHOSIS BY LABORATORY TESTS

Several problems arise in the differential diagnosis of cirrhosis. These are (1) the differentiation of cirrhosis without jaundice from other hepatomegalies; (2) the separation of cirrhosis with jaundice from hepatitis; (3) recognition of types of cirrhosis.

Recognition of Cirrhosis without Jaundice. The diagnosis of cirrhosis is suggested over other causes of hepatomegaly by the results of hepatic tests only if abnormalities in several of them indicate impaired hepatic function. Abnormal results of single tests occur in almost all hepatomegalies, and in the latent, or arrested, stage of cirrhosis all tests, with the exception of Bromsulphalein retention, may show normal results. In view of the unsatis-

factory results of the biochemical procedures, and of similar difficulties in clinical differential diagnosis, liver biopsy is recommended as the most reliable method for the diagnosis of cirrhosis. Removal of a larger specimen increases the reliability of the histologic method because of the lack of uniformity of the liver in cirrhosis.

Since nutritional cirrhosis is always diffuse, the probability of obtaining normal lobular tissue, as happens in postnecrotic cirrhosis, is small. One of two features should be present to establish beyond question the histologic diagnosis of cirrhosis and to differentiate it from periportal fibrosis, viz.: (1) septums connecting portal and central fields; (2) regenerative nodules. The recognition of nodules is sometimes difficult in small specimens if a part of the lobule partially separated by perilobular fibrosis simulates a nodule. Two features characterize the nodule:

1. The appearance of the hepatic cells in the nodule differs from that in the rest of the parenchyma; for instance, "plant" cells, or regenerative activity with two-cell-thick plates, are seen.

2. The hepatic-cell plates are directed toward a newly formed efferent venule in the center of the nodule, rather than toward the original central vein.

Cirrhosis with Jaundice vs. Hepatitis. In the differential diagnosis of cirrhosis with jaundice from acute hepatitis by laboratory tests, the former is indicated by relatively reduced thymol turbidity, especially in relation to the increased gamma globulin or zinc sulfate turbidity. Anemia is more common in cirrhosis than in hepatitis. The other tests are of limited value, except that low levels of serum albumin and cholinesterase early in the course of jaundice also indicate cirrhosis. Liver biopsy readily permits the differentiation.

Various metabolic disturbances cause structural and functional alterations of the liver, because excess metabolites are deposited in the liver. These storage diseases have been well known for many years, despite their rarity, because of the often dramatic changes produced in the liver and elsewhere. Nutritional fatty liver, probably the most important example of a metabolic injury, with storage of excess fat, has been discussed before (see Fatty Liver–Cirrhosis Syndrome from Malnutrition, Chap. 51). The separation of storage diseases caused by developmental or familial defects from those which develop as a result of some disease is often difficult, especially in the case of excess iron deposition.

Classification

Iron-storage diseases

Hepatolenticular degeneration (Wilson's disease)

Galactosemia

Glycogen-storage disease

Nutritional fatty liver (see Simple Nutritional

Fatty Liver–Alcoholic Fatty Liver, Chap. 51)

Lipid-storage diseases

Hepatic amyloidosis

IRON-STORAGE DISEASES

Excessive iron deposition, especially in the liver, is found in hemochromatosis, a condition in which skin pigmentation, diabetes, and cirrhosis form a syndrome called "bronzed diabetes." The disease is well known as a rare entity thoroughly described in the earlier literature [3035]. Originally the differentiation of hemochromatosis from harmless and common excess iron deposition, called hemosiderosis or, preferably, siderosis, appeared obvious, since siderosis was thought to be the result

of a redistribution of endogenous iron [1008]. The main problem of interest was the nature of the disturbance of the iron metabolism in idiopathic hemochromatosis. The differentiation between clinically significant hemochromatosis and insignificant siderosis has been complicated in recent years by the observation that a hemochromatosis-like picture develops in anemic patients receiving many blood transfusions. This has been called "transfusional siderosis" [1688, 3478, 3667, 3669], "secondary hemochromatosis" [306], or "exogenous hemochromatosis" [2967]. Furthermore, in South Africa a nutritional siderosis associated with cirrhosis was demonstrated [740, 1172, 1486]. The morphologic end results of these conditions may be indistinguishable from hemochromatosis [1008]. However, differentiation of the entities is needed for proper clinical management (Table 60).

Siderosis, or Hemosiderosis

In siderosis, excessive amounts of iron are found in many organs, particularly the liver, giving them a grossly apparent rust-brown hue. The presence of iron as such does not influence the appearance of the liver, except for the gross discoloration. Microscopically the excess iron appears as hemosiderin, i.e., as brown, well-defined pigment granules which give the prussian blue reaction (see Iron Pigments, under Pigment Granules–Cytoplasm, Chap. 3). Siderosis is either endogenous, because of excess release of iron from hemoglobin or other tissue constituents, or exogenous after parenteral or oral iron therapy or blood administration.

Pathogenesis. The lesion results from excess iron in the organism, but cellular iron metabolism is not altered, and no age or sex differences are found. Enlargement of the liver and spleen de-

depends on factors other than the increased iron content. The serum-iron level is usually elevated whenever the tissue stores of iron are increased [1008, 1486]. Hepatic-cell damage, as reflected by changes in the results of hepatic tests, is not caused by siderosis, and the iron-containing cells do not become necrotic.

IRON AS AN IRRITANT. Whether iron, as such, or some of its compounds acts as an irritant to hepatic epithelium or mesenchyma is not established [1996]. Administration of excessive amounts of soluble inorganic bivalent iron is poisonous, but the effect is metabolic rather than hepatotoxic [3279]. Ferric iron compounds given intravenously bypass the mucosal block of the duodenum and are taken up in the liver, mainly by the Kupffer cells. The little which reaches the hepatic cells is apparently innocuous. Excessive doses of saccharated iron oxide given intravenously produce

hepatocellular lesions [2445]. Thus a great excess of iron in the hepatic cells is toxic, or at least it makes the cells more vulnerable to other types of damage [847]. This excess is not found in siderosis. Many investigators have concluded that iron is inert and does not produce fibrosis [487, 1008, 3669]. Heavy iron deposition occurs without fibrosis in man [1172], as well as in animals [3669]. The length of time the iron is present is not important; it may remain in the liver many years without producing progressive fibrosis [334]. The injection into animals of the pigment isolated from human siderotic livers fails to produce fibrotic changes [1172]. Administration of large amounts of iron or blood to experimental animals does not uniformly produce hepatic fibrosis [1008]. The opinion that iron produces fibrosis, possibly associated with the destruction of hepatic cells, is primarily based on experiences with hemochromatosis [1008,

Table 6o Etiologic, Clinical, Functional, and Structural Features of Various Conditions Involving Increased Iron Storage

Feature	Siderosis				Hemochromatosis	
	Pigmented cirrhosis	Nutritional	Transfusional	Hemolytic	Primary idiopathic	Secondary, associated with anemia
Etiology:						
Disturbance of cellular iron metabolism.....	0	0	0	0	+	+
Excess exogenous iron.....	0	++	++	0	+	++
Independent hepatic injury.....	++	+	0	0	+	?
Clinical features:						
Age incidence.....	0	Increase with age	0	0	Fifth decade	0
Sex difference.....	0	Slight male predominance	0	0	Great male predominance	0
Skin pigment.....	+	0	±	0	++	+
Diabetes.....	0	0	0	0	++	+
Testicular atrophy.....	±	±	0	0	++	±
Decreased body hair.....	+	+	0	0	+	+
Jaundice.....	±	0	0	*	+	+
Laboratory features:						
Hepatic-cell damage.....	+	*	0	0	+	+
Increased serum iron.....	±	+	+	+	+	+
Iron absorption.....	N	+	N	N	+	+
Iron utilization.....	N	N	Down	Up	N	Down
Hepatic changes:						
Cirrhosis.....	++	*	0	0	++	+
Hepatic-cell iron vs. Kupffer-cell iron.....	N	LC > KC *	KC > LC	KC > LC	LC > KC	LC > KC
Cholangiolar iron.....	0	0	0	0	+	+
Connective-tissue iron.....	0	0	0	0	+	+
Size of iron granules.....	Fine	Fine	Fine	Fine	Large	Large
Iron in other tissues:						
Spleen.....	0	+	++	+	+	++
Intestinal villi.....	0	++	0	0	+	+
Pancreas.....	0	0	0	0	++	+
Marrow.....	0	+	+	+	±	+
Skin.....	0	0	0	0	++	+
Kidney.....	0	+	+	+	++	+
Kidney.....	0	0	0	0	++	+
Heart.....	0	0	0	0	++	+
Endocrine glands.....	0	0	0	0	++	+

Key: N = normal; 0 = absent; ± = minimal; + = moderate; ++ = severe; * = present but due to other causes.

1350, 2187, 2375, 2939]. However, even in siderosis some perilobular fibrosis is frequently found. Excess iron pigment probably acts as a mild stimulus for connective tissue proliferation.

DISTRIBUTION OF IRON. In most forms of siderosis the bulk of the hepatic hemosiderin is in the Kupffer cells as excess material. It is equally prominent in the hepatic cells only in nutritional siderosis. The ductules and bile ducts are free of iron, and little if any iron is found in the interstitial connective tissue. The total iron content of the liver is increased as much as ten to twenty times normal, to about 10 gm iron in the entire liver [3035]. The pancreas is free of excessive iron, and if fibrosis is present it has to be explained by other reasons. The intestinal villi are free of iron except in nutritional siderosis, indicating that in siderosis the distribution depends upon the route by which the iron enters the body [1008]. The skin also is free of iron and hemofuscin. In contrast, the kidneys and bone marrow contain much iron, as does the spleen, but the iron is not associated with much fibrosis. Hemofuscin and melanin pigment, found in large amounts in the skin in hemochromatosis, are absent.

Endogenous Siderosis. Siderosis is found in all conditions in which excessive red cell destruction occurs. This includes acute conditions, such as acquired hemolytic anemia or septicemia, and chronic conditions, such as chronic hemolytic jaundice, malaria, chronic passive congestion, leukemia, thrombocytopenia, pernicious anemia, and hepatic disorders such as cirrhosis. In sickle-cell disease, siderosis of the liver develops after adolescence when the spleen has shrunk in size [3171]. Siderotic pigment apparently also accumulates in hepatic cells after breakdown of intracellular iron-containing enzymes in mitochondria (cytosiderosis) [1172]. The increased hepatic iron in starvation has also been associated with the breakdown of either blood or tissue elements.

Exogenous Siderosis. PARENTERAL IRON OR BLOOD ADMINISTRATION. Experimental intravenous administration of iron compounds, particularly nonionizable ones, results in increased iron deposition in the liver, especially in the Kupffer cells [414, 487, 1008]. When ionizable iron compounds are injected, iron is deposited in the hepatic cells [2445]. Similarly some hepatic siderosis develops with hemosiderin, mainly in the Kupffer cells, after blood transfusions. This has been proved with radioautographic techniques in biopsy specimens [1008] of animals after intravenous administra-

tion of blood or hemoglobin, as well as in some patients who received repeated blood transfusions [671, 2375, 2967, 3667, 3669]. The siderosis is innocuous and has no clinical significance.

NUTRITIONAL SIDEROSIS. Administration of large amounts of iron to normal animals does not result in excessive iron storage because of a block to the absorption of iron in the intestinal mucosa [1247]. However, feeding of abnormal diets, such as corn grits and 2 per cent ferric citrate [1765, 3668], or a diet high in copper and choline [1704], or Tweens, produces progressive siderosis of the hepatic parenchymal and Kupffer cells with fibrosis. Siderosis is also associated with the nutritional defect which results from ligation of the pancreatic duct [1172] and from administration of alloxan [1466]. In certain forms of human malnutrition, especially in the Bantu of South Africa, siderosis develops [740, 1172, 1486]. The increased iron intake results from iron-rich foods and from iron cooking pots. Moreover, excess cytosiderotic iron may be released from hepatic-cell elements [1172]. Since the lesion is usually associated with cirrhosis, it has been identified with hemochromatosis [1172]. Some investigators have sharply separated it from hemochromatosis, claiming that the fibrosis may be an independent result of malnutrition, similar to those found without siderosis in other parts of Africa [408, 739, 1486]. Moreover the pattern of iron deposition and the clinical manifestations in typical cases differ from those of hemochromatosis [353] and resemble those of siderosis. In exceptional instances, possibly caused by additional factors, a picture similar to that of hemochromatosis develops.

Clinical Features. The incidence of the disease increases with age, and a slight male predominance is noted [1486]. Malaise is not outspoken. Skin pigmentation and decrease in body hair occur [1172], but other nutritional deficiencies may produce these changes. Hepatomegaly is found, whereas splenomegaly and testicular atrophy are not seen. Hepatic-cell damage, if present, also seems to be caused by other factors. Jaundice is not part of the picture, and diabetes mellitus is rare. The serum-iron level is usually elevated, and anemia is not present [1486].

Structural Alterations. Pathologically, hepatic changes range from fatty metamorphosis, hepatocellular degeneration, and necrosis to cirrhosis [1172, 1486]. The intensity of the siderosis is not related to the other hepatic lesions. Iron pigment is found in Kupffer cells and hepatic cells, depend-

ing on the stage of the condition, and the iron deposition in both locations can be very heavy. In severe siderosis, much iron is in the portal tracts, extracellularly and intracellularly, and sometimes even in the bile duct epithelium. The hepatic iron content ranges from a normal value of about 0.25 gm per liver to over 15 gm per liver. Hemofuscin is occasionally found in the liver. Much iron pigment is noted in the bone marrow, kidneys, and the intestinal villi. Little fibrosis and relatively little hemosiderin are present in the pancreas. The spleen contains even more hemosiderin than the liver, but fibrosis is not found. The heart and skin glands contain little if any iron pigment.

Pigmented Cirrhosis

Septal, or postnecrotic, cirrhosis is sometimes associated with lipofuscin deposition and siderosis from various causes, especially hemolysis. This results in findings difficult to differentiate from hemochromatosis, since diabetes and skin melanosis may sometimes be found in any type of cirrhosis, further complicating the differentiation [847]. However, the iron storage in this pigmented cirrhosis has no functional and clinical significance [1172, 1696], and iron absorption is not increased. The extent of cirrhosis formation far surpasses the deposition of hemosiderin, and, in contrast to hemochromatosis, the pigment is deposited mainly in Kupffer cells and other mesenchymal cells, not in the hepatic cells.

Hemochromatosis

About seven hundred cases of idiopathic hemochromatosis have been reported [52, 452, 847, 1853, 3035]. Almost 1.5 per cent of all diabetic patients supposedly suffer from hemochromatosis [356]. It occurs predominantly in males who are alcoholic, and it leads to clinical manifestations mainly in the fourth decade, although hypersideremia is detectable early in life and may be familial [755]. The disease occurs rarely in Negroes; it is also rare in women. Women with hemochromatosis are usually past the menopause or do not menstruate for other reasons.

Clinical Manifestations. The cardinal clinical symptoms are skin and mucous membrane pigmentation, hepatosplenomegaly, and diabetes. The clinical manifestations of cirrhosis, which include hepatomegaly, portal hypertension, ascites, and spider nevi, are found with varying incidence [52, 452, 1796, 1797, 3035]. Splenomegaly and esophageal varices are less frequent and severe than

in other forms of cirrhosis, while easy fatigability, reduction of body hair and sexual activity, and testicular atrophy are more severe. Not all symptoms and signs are necessarily present in each case. Melanosis and diabetes mellitus have been found in 80 per cent of the cases [3035]. In the pre-insulin era, over half the patients died in diabetic coma. The diabetes usually requires more insulin than other forms, but insulin insensitivity varies, and some patients tolerate the diabetes surprisingly well. With insulin treatment, life can be greatly prolonged [2215]. Death from ruptured esophageal varices occurs in about 5 per cent of the cases [3035]. Cardiac enlargement and low voltage in the electrocardiogram, with flattened or diphasic T waves, are common. Murmurs are usually absent, although blocks and arrhythmias occur. Heart failure is a frequent cause of death, with right ventricular failure predominating, particularly in those in whom the disease manifests itself early in life [354, 1797, 3271]. The failure responds poorly to therapy, and death usually occurs within 9 to 12 months of its onset. Endocrinologic manifestations may be prominent. The patients are very sensitive to stress and are poor operative risks. Abdominal pain and shock are present in some cases and have been associated with excess ferritin, which exerts a vasodepressing (VDM) effect [3306].

Laboratory Findings. The degree of liver function impairment depends upon the stage of the disease. In early stages, normal results of the flocculation tests are found in a high percentage of cases, and the albumin level and Bromsulphalein retention may also be normal. Serum gamma globulin is increased, although not to very high levels. As the disease progresses, the results of more and more tests become abnormal, and a fair correlation has been found between the results of the tests and the liver biopsy findings [52, 1797]. Bromsulphalein retention may remain normal despite the hepatomegaly. At the time of death, severe liver function impairment is usually noted. The glucose-tolerance curve is diabetic. The exocrine function of the pancreas is occasionally disturbed, and severe lipemia has been reported [1948]. The red blood count is usually normal, or a mild macrocytic anemia is present, but exceptional cases of secondary megaloblastic anemias have been reported. In these instances, the anemia has been assumed to be secondary to the cirrhosis and the hemosiderosis of the gastrointestinal tract [1846], but some features suggest

that these cases are examples of secondary hemochromatosis in pernicious anemia.

The fasting serum-iron level is usually elevated [270, 1008, 1063, 1180, 1552]. The iron-binding capacity of the serum is low. The saturation of the iron-binding serum proteins rises from a normal of about 30 per cent to over 90 per cent, but intravenous iron-absorption curves are flat [1180, 1181]. The high ratio of the disappearance rate of injected iron to the serum iron-binding capacity and the fact that the iron level after 2 hours is lower than the initial value in tolerance tests have been considered by some investigators as indications of increased tissue avidity for iron [1180]. The behavior of the fasting iron levels, as well as the high percentage of saturation of the iron-binding serum-protein, has been considered diagnostic for hemochromatosis [1181, 1552].

Morphologic Alterations. **MACROSCOPIC APPEARANCE.** The liver is usually enlarged, firm, and heavy. The surface and cut surface are rust brown in color and show various stages of diffuse septal cirrhosis with a hobnail appearance.

HISTOLOGIC CHANGES. All features of diffuse septal cirrhosis are seen, including fatty metamorphosis. The heavy accumulation of hemosiderin pigment, not necessarily uniform throughout the liver, is the most impressive finding. The hepatic cells are loaded with pigment granules. If the hepatic cells are intact, less hemosiderin is found in the Kupffer cells. In some instances an iron-free hemofuscin, demonstrable by the PAS reaction, is found in the Kupffer cells and other mesenchymal cells, especially in the smooth muscle. Iron pigment is also present in the epithelium of bile ductules and ducts. Periportal ductules and the smallest bile ducts appear increased in number. Coarse iron pigment granules are found in the portal tracts in the wandering cells, as well as extracellularly (Fig. 175, upper right). This may be associated with radiating portal fibrosis, giving the impression that iron impregnation of connective tissue acts as a stimulus for fibrosis (Fig. 175, lower left). In more advanced stages, septums form and appear as thick sheets connecting portal tracts with each other and with the central fields, thereby dissecting the lobules, and regenerative nodules form. However, the regenerative processes are less conspicuous than in other types of septal cirrhosis. In this stage the connective tissue distribution is similar to that of diffuse septal cirrhosis except that the septums are thicker and more hyalinized in the later stages than in nutritional septal cir-

rhosis (Fig. 175, lower right). Hepatic cells which are heavily laden with iron pigment granules disintegrate, either because of the pigment content itself [2187] or for independent reasons, such as infections or acute malnutrition, which occur in all forms of cirrhosis. In the areas of hepatocellular necrosis, the Kupffer cells become very rich in hemosiderin, which is drained to macrophages in the portal tracts. Under such circumstances, the predominance of hemosiderin deposition in mesenchymal cells, rather than in epithelial cells, may simulate the findings in siderosis [847]. This differential diagnostic difficulty is easily overcome if nonnecrotic areas are studied. In patients dying in hepatic coma, centrilobular necrosis and severe bile stasis are usually present. Ischemic necrosis caused by arteriosclerosis has also been reported [1858].

CARCINOMA FORMATION. Proliferation of ductules with iron-laden epithelium is often associated with multicentric hepatic tumors, either cholangiomas, hepatomas, or both. The absence of iron pigment from carcinomatous structures differentiates them from regeneration better than other cytologic characteristics. Development of primary carcinoma of the liver occurs in about 20 per cent of the cases of hemochromatosis [3492], and the incidence of carcinoma is greater in other types of cirrhosis. Not all investigators agree with this observation [3035]; which is in accord with our own experiences. Carcinoma formation is not related to the duration or severity of the hemochromatosis, as judged morphologically, although it occurs more often when the disease manifests itself later in life. The clinical diagnosis can be made by liver biopsy and by the presence of rapid enlargement and nodularity with pain, rapidly recurring ascites, skin changes, and hypoglycemic attacks [233].

CHEMICAL ANALYSIS. The iron content of the liver, as well as of the entire body, is greatly increased; the liver may contain 25 gm, or about 50 times the normal amount. In addition, other metals, such as calcium, copper, and lead, are found in increased amounts in the liver [3035].

BIOPSY FINDINGS. Liver biopsy permits the recognition of early stages [52, 210, 3349]. Coarse iron granules are found in hepatic cells, and also some in Kupffer cells. Fibrosis may be minimal (Fig. 175, upper left). The prevalence of the hemosiderin deposition in hepatic cells permits the differentiation from siderosis. This characteristic may disappear in progressive cirrhosis with hepatic-cell damage, and therefore biopsy findings

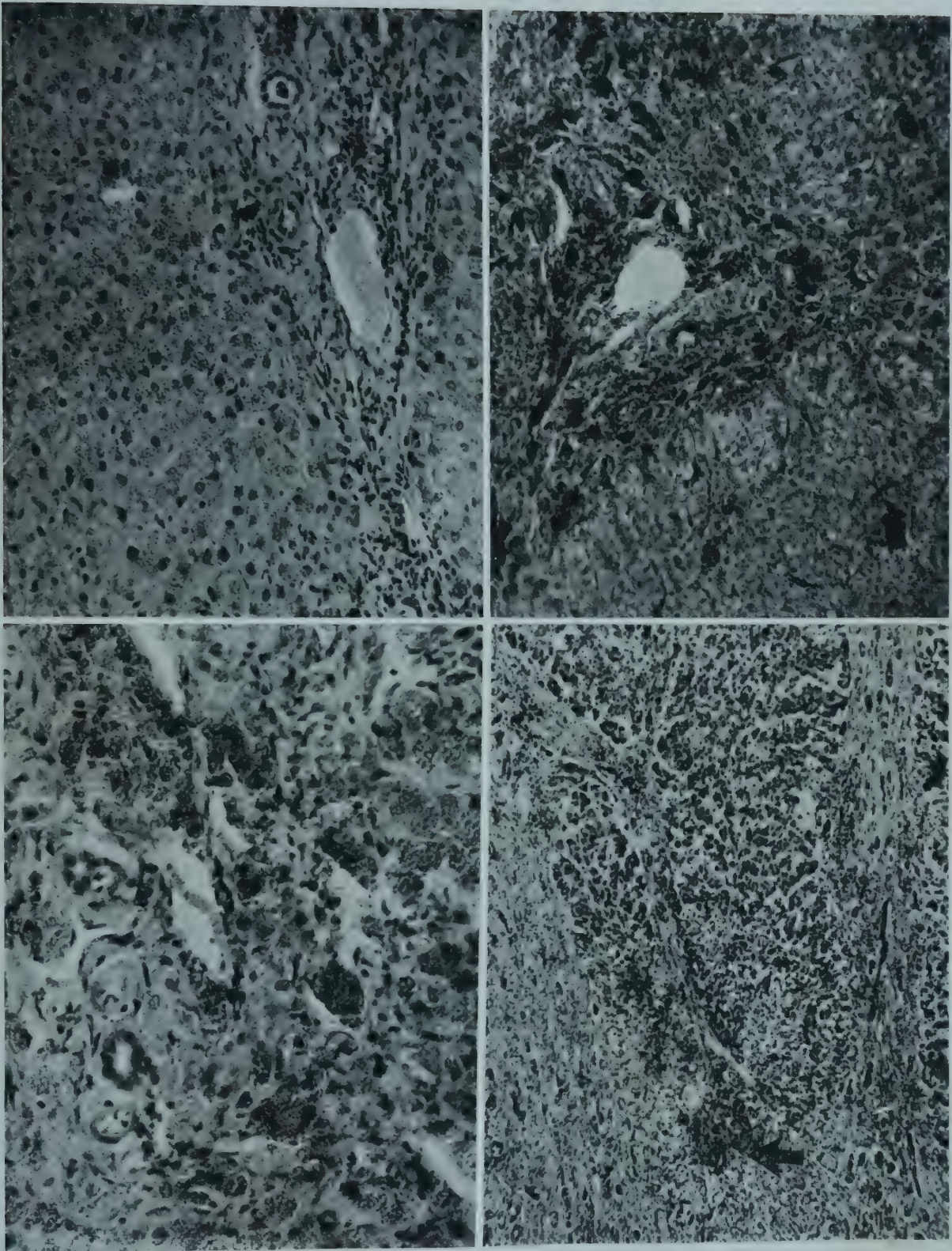


FIG. 175 Hemochromatosis. *Upper left.* Biopsy specimen of early stage. Iron granules in hepatic cells and some in Kupffer cells. Portal fibrosis and inflammation. H&E ($\times 125$). *Upper right.* Biopsy specimen of further advanced stage. Heavy iron deposition in the hepatic cells, in Kupffer cells, and in the portal tracts within and outside of cells. The portal tracts are enlarged and stellate in shape. H&E ($\times 100$). *Lower left.* Portal tracts of specimen in upper right, showing iron pigment in hepatic cells, ductular cells, and mesenchymal cells, and also extracellular iron deposition. *Lower right.* Cirrhosis with wide septums rich in ductules. Iron accumulation in hepatic cells and bile duct cells and especially extracellularly in connective tissue (arrow). H&E ($\times 88$).

are more diagnostic in early or quiescent stages [847].

CHANGES IN OTHER ORGANS. Hemosiderin is seen in excessive amounts in pancreas, skin, heart, muscle, anterior pituitary, adrenal cortex, thyroid, parathyroid, salivary glands, gastric and duodenal mucosa, and choroid plexus. The intestinal villi contain small amounts. Lymph nodes, such as the portal nodes, which drain organs rich in iron, show siderosis. The testes are pigmented, but the atrophy is probably secondary to pituitary changes. In addition to hemosiderin, hemofuscin (see Hemofuscin, under Cytoplasm, Chap. 3) is deposited in excessive amounts, especially in smooth-muscle fibers of blood vessels and the heart. It is also noted in the pancreas, in which hemosiderin is deposited in the acinar, ductal, and islet epithelium, as well as in the fibrosing connective tissue. This is associated with excessive diffuse fibrosis. In the skin, four different pigments, found in varying amounts, account for the bronzed-slate hue characteristic of hemochromatosis. These are (1) hemosiderin in the corium, especially within the sweat glands, and little in the epidermis; (2) lipofuscin in smooth muscles of the blood vessels and in the lamina propria of sweat glands; (3) melanin in the epidermis in increased amounts, as in any other type of cirrhosis; (4) bile pigment, poorly demonstrable histologically. Iron pigment in the kidney is found mainly in the loops of Henle, in contrast to siderosis, in which it is found in the proximal convoluted tubules [3478]. The spleen shows only a moderate increase in iron content, and splenic abnormalities are mainly the result of cirrhosis. The bone marrow contains little hemosiderin.

Hemochromatosis vs. Hemosiderosis. Hemochromatosis differs morphologically from siderosis by (1) a greater iron content in the liver, pancreas, and adrenal and pituitary glands; (2) a lesser iron content in the spleen and lymph nodes; (3) almost complete absence of iron in the bone marrow; (4) the presence of hepatic cirrhosis, while in siderosis periportal fibrosis is present, or, if cirrhosis is present, it is caused by other factors; (5) more pronounced pancreatic fibrosis.

Etiology. The cause of primary hemochromatosis is as yet not established, and therefore the term "idiopathic" is applied to it.

VARIOUS THEORIES. Although most authors assume that an inborn error of iron metabolism is responsible for hemochromatosis [3035], many other theories have been presented to explain the lesion.

Some are (1) alterations of reticuloendothelial function, in the sense that these cells are unable to return iron to the circulation [943, 2939, 3414]; (2) increased permeability of the hepatic sinusoids for red cells, which are subsequently removed by phagocytes [2797]; (3) altered innervation from the midbrain centers [396, 2730]; (4) cirrhosis as the primary disease [1814], assuming that hepatic-cell injury precedes the iron deposition or is an independent consequence of the same toxic factor causing the iron deposition; (5) disturbance of ferritin metabolism in the liver [823, 1247], although no chemical evidence for this has been found [1248]; (6) increased intestinal absorption of iron owing to lowering of the mucosal block in the intestine [1247, 1559], but no defect in ferritin formation in the duodenal mucosa was demonstrated [1248]; (7) a defect in the complex regulatory mechanism of the serum-iron level connected with increased saturation [1180] or other changes in the iron-carrying beta globulin (siderophilin) [823].

The theories of intoxication implicating such factors as alcohol abuse, bacterial toxins [2187], or copper poisoning, are now largely discarded. Experimental copper administration does not produce hemochromatosis.

SHELDON'S THEORY. At present Sheldon's theory of an inborn error of the iron metabolism appears most acceptable [3035]. This defect is present in all the cells of the body and causes an increased avidity of all tissues for iron through the duodenal mucosa, demonstrated by increased absorption of radioactive iron [353, 1247]. Also excessive deposition of iron-containing pigments occurs in various organs, especially those more important in iron metabolism, like the liver. This phenomenon is reflected in the rapid decrease of serum iron after intravenous administration [1181]. The simultaneous accumulation of non-iron-containing pigments, such as hemofuscin, is not explained. In view of the limited facilities of the body for iron excretion, a slight increase in daily iron absorption, too small to be recognized in balance studies, may lead to an accumulation of the iron increments through the years. Eventually excessive iron deposition causes increasing fibrosis because of the slight irritative action of iron pigment. Cirrhosis begins relatively late in life, usually after the fourth decade. The excessive iron deposition in the hepatic cells probably produces or facilitates hepatocellular necrosis. Additional factors probably contribute to the cirrhosis.

formation but are not necessary. The toxic effect of the iron pigment itself is reflected in the improvement in the patient's condition after drainage of iron from the body, by phlebotomy or chelating agents. Iron salts supposedly interfere with enzymes responsible for the formation of pyruvate and consequently with the re-formation of ATP, the tissue energy source. This mechanism may be more significant in the heart than in the liver and has been held responsible for the cardiac enlargement and failure. The disturbance of cellular iron metabolism is supposedly present throughout life, but the iron residues do not become large enough to produce significant tissue alteration until middle age. The lower incidence of hemochromatosis in women is explained by iron loss during menstruation and by lactation. Heredity is a factor in the congenital defect, and a familial incidence has been claimed [755, 1797, 2045, 2800].

In conclusion, three factors are necessary for the development of hemochromatosis (1) entrance of excessive amounts of iron into the body, owing to increased intestinal absorption; (2) entrance of excessive amounts of iron into hepatic parenchymal cells; (3) a slowly acting irritating effect of iron pigment, causing fibrosis and cell death.

Laboratory Diagnosis of Hemochromatosis. The diagnosis of hemochromatosis can be confirmed by determining the serum-iron level, and particularly the iron-binding capacity of serum proteins, and by iron-tolerance tests [1063, 1180, 1552, 1559] or by histologic methods. In liver biopsy specimens [52, 1762, 1796, 1797, 3349] hemosiderin is found in a more or less cirrhotic liver, more of it in hepatic cells than in Kupffer cells, and in ductules and the connective tissue. In necrotic areas mesenchymal hemosiderin may predominate [847]. Hemofuscin is not necessarily present. Iron pigment can be demonstrated in the gastric mucosa by gastroscopic biopsy [52]. Skin biopsy has been widely used. Hemosiderin in epithelial cells in urinary sediment does not differentiate hemochromatosis from siderosis.

Treatment. The excess iron in hemosiderin can be drained into the circulation for utilization in hematopoiesis [627], resulting in arrest or improvement of the patient's condition. Repeated phlebotomies reduce the stored iron, as shown in liver biopsy specimens [742, 743, 1559, 1797, 2577], and improve hepatic function, glucose tolerance, the appearance of the skin, and the clinical condition [743, 3496]. An average of 25 to 45 liters of blood is withdrawn yearly, representing

12 to 20 gm iron [743], and the red cell count is kept between 3.5 and 4.0 million per cubic millimeter. Chelating agents, organic compounds which form strong nonionic water-soluble combinations with metal ions, such as disodium calcium versenate, have been used in attempts to mobilize iron in a form which can be excreted in the urine [3637]. The value of such therapy and the dangers of side reactions remain unestablished. The treatment of the diabetes and cirrhosis is symptomatic.

Secondary Hemochromatosis Associated with Anemia

In recent years, an increasing number of reports describe patients who received blood transfusions and who developed hemochromatosis. In these reports siderosis is frequently not clearly separated from hemochromatosis, although some patients eventually exhibit the classic triad of hemochromatosis, viz., pigmentation, diabetes, and cirrhosis.

Pathogenesis. ROLE OF BLOOD TRANSFUSIONS. Since many of these patients received many blood transfusions, originally the excess iron introduced with the blood was considered to be the main cause of so-called "secondary hemochromatosis" [1688], "exogenous hemochromatosis" [2967], or "transfusional siderosis" [3669]. However, cases have been reported in which the hepatic iron was up to 75 gm [3669], considerably exceeding the iron in the blood transfusions, which contain about 250 mg per pt [671, 2375, 3064, 3669]. In some patients only a few transfusions were given before the diagnosis of hemochromatosis was established by liver biopsy [306]. Hemochromatosis developed in one patient with thalassemia who had no transfusions. The transfused blood, therefore, appears to be merely a contributing or aggravating, rather than a causative, factor. Prolonged oral iron therapy in the presence of anemia may also be responsible for the lesion [1205, 3472], especially in those patients who have not received a sufficient number of transfusions to account for all the excess iron, even if the normal 10 to 15 mg of dietary iron per day is considered completely absorbed.

ROLE OF REDISTRIBUTION OF IRON. In all cases reported (some apparently only hemosiderosis), anemia was a prominent feature, e.g., prolonged hemolytic anemia [306, 671, 2375, 3064, 3472], anemias with hemolytic components such as pernicious anemia [2375] or sickle-cell anemia [1108, 3171], refractory or aplastic anemia [306, 1688, 2375, 2967, 3701], erythremic myelosis, and vari-

ous forms of leukemia or thalassemia (Cooley's anemia) [703, 918, 1561]. In all these primary anemias, hemoglobin formation does not keep pace with the amount of iron in the body, and hepatic siderosis occurs, owing to redistribution of iron. However, the amount of iron involved is small, and the same phenomenon occurs in secondary anemia; for instance, following chronic glomerulonephritis [2878, 2967]. Therefore, redistribution of iron is only a contributing factor, not a causative one.

ROLE OF ANEMIA. In anemia of any kind, iron absorption from the intestine is increased [306], and the mucosal block created by the ferritin mechanism is lowered, possibly because the oxygen deficit favors the existence of the more soluble bivalent iron salts [1247], or because more of the intestine actively absorbs iron. The results of iron-tolerance tests in thalassemia, acute leukemia, aplastic anemia, and pernicious anemia in relapse are similar to those in primary hemochromatosis [1180]. Anemia itself therefore increases the intestinal absorption of iron from a normal of 1 mg a day, thus creating conditions similar to those of hemochromatosis and favoring its development if the patient lives long enough.

However, while siderosis regularly develops in chronic primary anemia, the incidence of secondary hemochromatosis is low. This suggests an additional factor which favors the development of hemochromatosis. Severe anoxia has been suggested [2967], and iron storage is increased in lowered oxygen tension [1336]. However, iron metabolism is not disturbed in chronic anoxia without anemia. Nonspecific damage to the hematopoietic system and the tissues in general, including the liver, has been considered a causative factor in aplastic anemia [3701], although similar changes occur in other types of anemia. Prolongation of life by blood transfusions in otherwise fatal conditions has been considered a cause of metabolic aberrations [3669]. Viral hepatitis may develop as a result of the blood transfusions and may further modify the iron metabolism. The basic factor which accounts for the transition of hemosiderosis into hemochromatosis in anemia requires, however, further elucidation.

In secondary hemochromatosis associated with anemia the same factors are present as in primary hemochromatosis, namely the entrance of excessive amounts of iron into the body, excessive deposition of iron pigment in the hepatic cell, and the irritating effect of iron pigment. The excess iron

enters the body much more rapidly than in primary hemochromatosis, because anemia lowers the intestinal barrier and because of therapeutic administration of blood or iron. Therefore secondary hemochromatosis can occur at any age and occurs equally in males and females. In contrast to idiopathic hemochromatosis, which takes several decades to develop, the hepatic changes in secondary hemochromatosis develop within a few years, about 9 months being the most rapid on record, and cirrhosis in secondary hemochromatosis is actively progressing and usually minimal in extent.

Clinical Manifestations. Secondary hemochromatosis presumably is present if one of the signs of primary hemochromatosis, such as pigmentation or diabetes, appears in a patient with severe anemia, the patient usually being one who has received multiple blood transfusions or intensive antianemia therapy. Only exceptionally do the clinical symptoms referable to this condition become of major importance, and the cause of death is almost always the underlying cause of the anemia. Recovery has been reported, with disappearance of preexisting pigmentation and ascites [2476].

Structural Alterations. Primary hemochromatosis and secondary hemochromatosis associated with anemia are structurally similar, except that the changes in the secondary form are less severe. Moreover the iron distribution in secondary hemochromatosis is intermediate between that in siderosis and that in primary hemochromatosis. In the secondary form, much iron pigment is deposited in the primary sites, such as the hepatic cells, pancreas, skin, myocardium, and endocrine glands, but not so much as in primary hemochromatosis, and much is found in the Kupffer cells and in the spleen and bone marrow, as in siderosis. In secondary hemochromatosis cirrhosis formation has begun, in contrast to siderosis in which lobular fibrosis, at most, is present. The diagnosis has been made in liver biopsy specimens [306] (Fig. 176). At the time of death, cirrhosis formation is never so much advanced as in primary hemochromatosis.

HEPATOLENTICULAR DEGENERATION (WILSON'S DISEASE)

Hepatolenticular degeneration, or Wilson's disease, is characterized by the involvement of the extrapyramidal basal ganglion system with cirrhosis of the liver and pigmentation of the margin

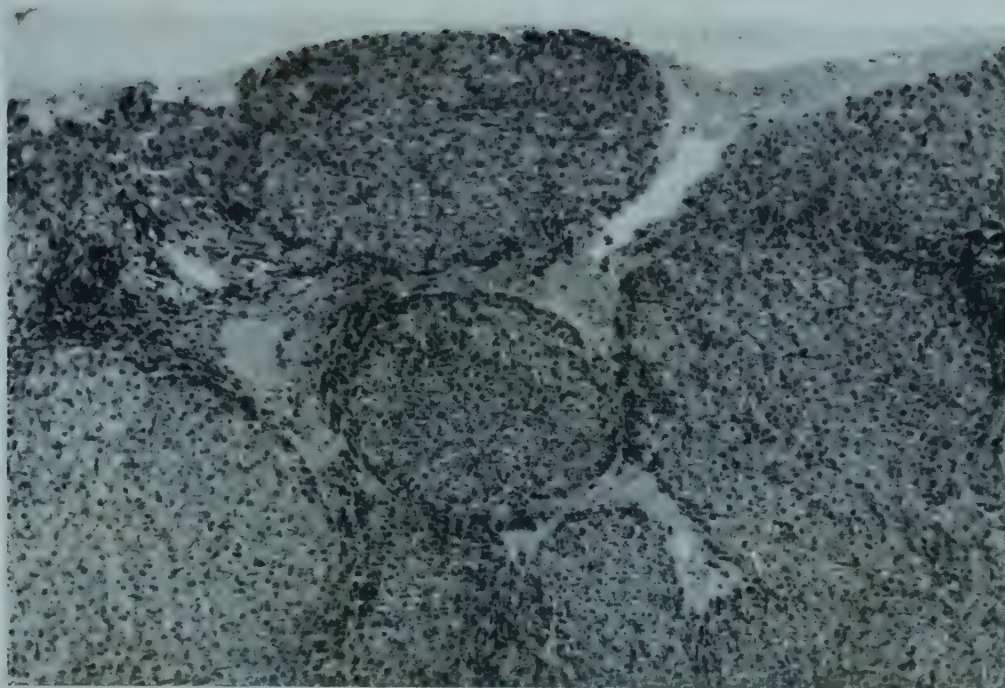


FIG. 176 Biopsy specimen from patient with secondary hemochromatosis and protracted refractory anemia, revealing cirrhosis with hemosiderin deposition in Kupffer cells, and less deposition in hepatic cells. H&E ($\times 72$).

of the cornea of the eye (the Kayser-Fleischer ring). A fairly acute form, typical Wilson's disease, develops in the preadolescent age group, and a chronic form, pseudosclerosis of Strümpell-Westphal, is seen in young adults. In addition, forms occur in which not all the cardinal symptoms are present. For instance, the abdominal form of Kahrer shows the Kayser-Fleischer ring and the metabolic changes but no neurologic alterations. Idiopathic juvenile aminoaciduria is asymptomatic, liver function is normal, and no Kayser-Fleischer ring develops. It occurs in siblings of patients with other forms and sometimes progresses into one of the other forms [3400].

Etiology. The disease is familial [194, 3400] and seems to be inherited as a recessive trait after high consanguinity rates in the parents. Three biochemical defects have been found, aminoaciduria, increased urinary excretion of dicarboxylic amino acid peptides, and disturbed copper metabolism, which are the probable causes of the hepatic impairment [194, 768, 2246, 3157, 3189, 3398].

DISTURBANCE OF COPPER METABOLISM. The plasma normally contains about $100\text{ }\mu\text{g}$ copper per 100 ml. The bulk of this is in the form of a stable, indirect-reacting compound, ceruloplasmin, the protein moiety of which is alpha globulin. Only a small portion of plasma copper is direct-reacting and loosely bound to albumin, or else it is not bound to protein at all and therefore easily diffusible. In addition, increased tissue avidity for

copper seems to be present. The basic abnormality in Wilson's disease is an inborn defect in ceruloplasmin formation [196, 509, 2914]. The plasma-copper level is reduced to less than half of normal, with an increase in the direct-reacting fraction [194, 196]. This is responsible for increased absorption of dietary copper [196, 769] and an excessive excretion of copper in the urine, excretion rising from a normal of $0.5\text{ }\mu\text{g}$ to 200 to $700\text{ }\mu\text{g}$ a day [194, 698, 768, 2246, 3157]. Despite the excess urinary excretion, the patients remain in positive copper balance [509]. The presence of copper in an active and diffusible form in the plasma facilitates high concentrations of copper in the spinal fluid and in the tissues, including the brain, liver, kidney, and the corneal ring [509, 698, 768, 2246, 2656, 3157]. Damage to these organs by the excess copper deposition has been assumed but so far not proved. The excess urinary copper excretion leads to the formation of copper-peptide complexes, or chelation. These complexes are thought to interfere with the tubular reabsorption of amino acids [3399], and this explains the combined excessive excretion of amino acids and copper in the urine and the increase in copper excretion after protein intake [194, 196, 2246, 3399].

AMINOACIDURIA. Aminoaciduria is persistent and involves all amino acids in the plasma [194]. It is not associated with significant alteration of the serum-amino acid or serum-protein level, nor does

the total protein intake exert a recognizable influence [3189]. Aminoaciduria occurs in the fasting state but is increased by the ingestion of glycine or alanine [2246, 3399]. Renal glycosuria occurs in some of the patients [650, 3400]. The severity of the hepatic damage is not reflected in the degree of the aminoaciduria, nor does the duration of the disease appear to be of importance. The excess amino acid excretion was considered to be the basic defect, especially since it may precede any other manifestation, and the endogenous amino acid deficiency was thought to lead to cirrhosis, analogous to cirrhosis in Fanconi's syndrome [551, 770].

PEPTIDURIA. Disturbed metabolism of dicarboxylic amino acid peptides, as a result of some defect in tissue proteolytic enzymes, may lead to excess formation of copper peptide complexes, which are then excreted in the urine [3398].

Hepatic Involvement

Although mental disease dominates the picture in almost all instances, a characteristic alteration of hepatic function and structure occurs.

Clinical Manifestations and Laboratory Findings. Clinical manifestations of hepatic dysfunction, such as jaundice, hepatomegaly, hematemesis from esophageal varices, spider angiomas, and splenomegaly [1069, 1087, 1471, 1540, 2246, 2684], are associated with abnormal results of hepatic tests, including increased cephalin flocculation and thymol turbidity, Bromsulphalein retention, and hyperbilirubinemia [1069, 1471, 1540]. In patients without clinical manifestations of hepatic injury, the hepatic tests often yield abnormal results [1069, 3157, 3275]. The neurologic manifestations usually precede the hepatic changes, although onset of the disease with hepatic symptoms has been reported [1069, 1471]. Hepatic failure and related manifestations are the cause of death in about half the cases in some series [1069], and in less than half in others, where infections predominate.

Structural Alterations. Fatty metamorphosis and portal fibrosis are seen in liver biopsy specimens [2912, 3398]. At necropsy, cirrhosis is found, varying in type, degree, and activity [943, 1069, 1087, 2684, 3157]. Most cases show some features of postnecrotic cirrhosis [943, 1069, 2797, 3157], with large nodules composed of several lobules separated by broad bands of collapsed hepatic framework. Occasionally this appears like the coarse nodular hyperplasia of Marchand (see Mac-

roscopic Appearance, under Postnecrotic Cirrhosis, Chap. 44). Often the process initiating the cirrhosis has long since burned out and is reflected by a history of antecedent jaundice [3157]. The limiting plate appears sharply outlined; hepatic-cell damage is often absent. Portal hypertension with splenomegaly is sometimes in the foreground [1087, 2684].

Central Nervous System Involvement. In fully developed forms, especially acute ones, bilateral and symmetrical involvement of the putamen is present. It becomes darkly discolored and contains minute cysts. Occasionally this involvement extends to the globus pallidus and to the internal and external capsule. Sometimes a cribriform state of the frontal lobe cortex and of the dentate nucleus is seen. Microscopically, the ganglion cells show degeneration and atrophy, and finally they disappear. This is associated with glial proliferation without vascular or inflammatory manifestations.

Clinically a progressive tremor and stereotyped facial expression and finally mental deterioration are noted. Rapidly progressive acute forms have been reported.

Therapy. A low-copper diet is not practical, but the addition of potassium sulfide to the diet has been suggested to prevent absorption of copper. A high-protein diet or casein hydrolysate supplements assist in the removal of excess copper via the urine. Increased removal of copper by BAL improves the central nervous system manifestations [196, 698, 768, 2912]. BAL, chelating agents (versenes), and ion exchange resins appear to be the most promising therapeutic agents. ACTH is without effect [196].

GLYCOGEN-STORAGE DISEASE

Glycogen-storage disease is a disturbance of carbohydrate metabolism in which excessive deposition of glycogen in the organs is the predominant finding. The disease is familial, is seen in young children [2234, 3407], and occurs in siblings [3354]. The classic description of Von Gierke linked his name with this disease, although it was first described by Snapper and van Creveld in 1928. A cardiac type is usually separated from a hepatic type.

Pathogenesis. In Von Gierke's original cases, hepatic glycogen did not decrease after death so rapidly as that in the normal liver, while addition of normal tissue resulted in its rapid degradation.

[2952]. This suggested a defect in the glycogenolytic enzyme system. A specific enzyme, glucose-6-phosphatase, has been shown to be absent in severe cases, and its activity is reduced in mild cases [749]. The glycogen itself appears normal in most instances, but in a few cases its molecular arrangement was found to be abnormal [1045, 1586]. Consequently excess glycogen is found in the liver without other significant structural hepatic changes. Reduced release of glucose from the liver, as well as inability of the glycogen-laden liver to take up glucose, renders the blood-sugar level unstable, and hypoglycemic and hyperglycemic episodes are frequent. Increased fat and protein utilization results in wasting and retardation of growth.

Glycogen Storage in the Heart. Cardiomegaly is prominent when much glycogen is deposited in the myocardium [800]. Fatal cardiac failure usually occurs in the first year of life. The hepatic glycogen level is also elevated in this form.

Glycogen Storage in the Liver

Clinical Features. A few hundred instances of this disease have now been described [399, 1045, 1905]. Children with this condition are susceptible to infections to which they rapidly succumb, so that few have survived to adolescence [951], and apparently only one to adulthood. Hypoglycemic convulsions are frequent, and the patients are extremely sensitive to insulin. These facts help in the differentiation from juvenile diabetes, with which this condition may be confused. Hepatomegaly is often present at birth without splenomegaly. The large left lobe may sometimes be mistaken for the spleen. Some degree of physical retardation, with dwarfing and obesity and occasional mental deficiency, is present.

Therapeutically, low-carbohydrate-high-protein diets have been recommended to increase the supply of blood sugar from sources other than hepatic glycogen [2076]. ACTH has been used to prevent the fasting hypoglycemia and ketonuria [1905].

Laboratory Findings. Glucose-tolerance curves vary but have a tendency toward a diabetic type, with glycosuria reflecting impaired hepatic glycolysis [1905, 2076]. Very high levels of blood glycogen are found. Hypoglycemia with ketonuria rapidly develops when food is withheld, and the blood-glucose elevation is minimal or absent after subcutaneous epinephrine administration. Blood-cholesterol level is often elevated [1045, 1905, 2076], and lipemia has been reported [1045].

Only few reports on the results of hepatic tests are available [399, 1045, 1905, 3117, 3354]. Abnormalities may be entirely absent, although results of cephalin flocculation, Bromsulphalein retention, prothrombin time, icterus index, and serum-alkaline phosphatase activity are occasionally abnormal.

Structural Alterations. The liver is greatly enlarged, pale, and glassy in appearance, with a smooth surface. In biopsy [399, 1045, 1577, 1905] and autopsy specimens, the hepatic cells are enlarged and contain very large amounts of glycogen, giving the cells a vacuolated appearance in routine paraffin sections. Similar large amounts of glycogen are rarely seen in other conditions except hyperinsulinism. The Kupffer cells occasionally contain glycogen. The chemical glycogen content exceeds 10 to 15 gm per 100 gm wet tissue. In patients who survive for a few years, perilobular fibrosis develops [1045, 1752], supposedly as a result of disturbed lobular circulation [551]. Multiple hepatomas were found in one patient who died at ten years of age [2234]. Inflammatory changes are not found. The kidneys are usually enlarged and also contain increased glycogen.

GALACTOSEMIA

Galactosemia (galactemia or galactose diabetes) is a rare inborn error of galactose utilization. Its occurrence in siblings [207] and members of the same family [815] indicates the hereditary nature of the disease, apparently as a recessive trait.

Pathogenesis. Many partially conflicting theories have been presented for the basis of the metabolic error [207, 885, 1202, 3352]. It is most probably a defect in the specific hepatic enzyme reactions engaged in the transformation of galactose-1-phosphate to glucose-1-phosphate, via the intermediate galactose phosphate combined with the nucleotide, uridyl. Among the known enzymes are galactokinase and the Walden enzyme, phosphogalactoisomerase. The galactose accumulation in the blood is cytotoxic, especially to the lens, producing cataracts, and probably to the brain and kidney [2777]. Moreover aminoaciduria develops, possibly because of competition for tubular reabsorption [1538]. The blood-glucose level is frequently reduced. The hepatic injury is not caused by excess storage of galactose in the liver [2234], but rather by metabolic imbalance resulting from faulty carbohydrate utilization and the urinary

loss of amino acids. Thus, as far as the hepatic consequences are concerned, it is a form of endogenous malnutrition. The defect persists throughout life but loses its functional significance as the galactose intake in the milk becomes a less prominent part of the diet.

Clinical Manifestations. Changes are noted a few days after birth and become more outspoken as the infant becomes older, subsiding after about five years of age. Hepatomegaly is encountered, and the spleen is sometimes enlarged because of portal hypertension. Evidence of malnutrition and cataracts, mental retardation, and edema are frequently noted [207, 815, 1202, 1215, 1905, 2260, 2777]. On the commonly used milk diet, galactose is excreted in the urine and albuminuria is frequent. The blood-glucose level is low, but the glucose-tolerance curve is normal or diabetic in type on a milk diet, while on a lactose-free diet, results of glucose, insulin, and epinephrine-tolerance tests become normal. A galactose-tolerance test shows prolonged high blood levels of galactose simultaneously with depression of blood-glucose level [1202, 1215, 1905]. In some instances hepatic function is altered, as reflected by abnormal cephalin flocculation, prolonged prothrombin time, and Bromsulphalein retention [1905], but in other instances the changes are less apparent [815, 1271] and jaundice is exceptional. Removal of milk or other sources of galactose from the diet restores health, with regression of cataracts and of hepatic symptoms [1202].

Structural Alterations. Biopsy [3352] or autopsy [207] specimens of the liver show fatty metamorphosis, with transitions into diffuse septal cirrhosis similar in appearance and development to the fatty liver owing to malnutrition [3352]. Large regenerative nodules rich in glycogen have been reported [885]. Removal of galactose arrests and even improves the hepatic lesion.

Varieties of Hepatic Glycogen-storage Disease. In addition to the typical form of glycogen-storage disease in the liver, two other, apparently rarer, forms have recently been recognized [2725]:

1. Diffuse glycogenosis with hepatic cirrhosis, in which symptoms, mainly referable to hepatic involvement, appear in late infancy. In addition to cirrhosis, glycogen deposits in phagocytic cells are found in the spleen, lymph nodes, and the intestinal mucosa. The lesion is considered to be the result of an abnormal configuration of glycogen.

2. Glycogen storage in liver and muscle. This

is very rare, appearing late in infancy. It is associated with another type of alteration of hepatic glycogen.

LIPID-STORAGE DISEASES IN THE LIVER

The metabolic disorders in the relatively rare lipid-storage diseases have in common the deposition of lipid material in the liver [3317]. However, this deposition has relatively little effect upon hepatic function, and in this sense these disorders are not hepatic diseases.

Gaucher's Disease. Gaucher's disease results from abnormal deposits of kersin in the reticulo-endothelial elements of the hematopoietic system and the liver. This cerebroside is normally found chiefly in the brain. It is not carried in the blood, and its abnormal deposition in reticulum cells and histiocytes has been described as a deviation of intracellular metabolism [2504]. The familial disorder makes itself apparent in childhood, but since it does not interfere greatly with life expectancy it can be found in all age groups. An acute form occurs in infants [1, 2504].

The predominant findings are hepatosplenomegaly, skin pigmentation, and lymphadenopathy, with slight anemia, thrombocytopenia, and leukopenia as an expression of bone marrow involvement [2732]. Liver biopsy, in addition to bone marrow and spleen biopsy, is useful as a diagnostic procedure [2732]; demonstration of Gaucher's disease was one of the early findings of liver biopsy. The results of the hepatic tests are usually not significantly altered. Indirect-reacting bilirubin and Bromsulphalein retention may be increased. Jaundice and severe disturbance of hepatic function with ascites have been reported in exceptional cases [2353].

The liver is usually very large, weighing up to 4,000 gm [2732], but the degree of enlargement is not so great as that of the spleen [2732, 3317]. It is firm and smooth, and, on histologic examination, the transformation of Kupffer cells and other mesenchymal cells to Gaucher cells and the presence of these cells in the portal tracts are seen to produce a characteristic picture. The Gaucher cell is a large, usually polygonal cell with a small eccentric nucleus. Sometimes several nuclei are found, and multinucleated giant cells are seen. The cytoplasm is opaque and has a fine fibrillar meshwork. Vacuoles, if present, are probably artifacts. The cytoplasm does not take fat stains but is stained with Mallory's aniline blue. Kersin is

demonstrated histologically by the periodic acid-leukofuchsin method [2356]. The Gaucher cell sometimes contains iron pigment. Proliferation of these cells results in nests which distort the lobular architecture [2353] and cause perilobular fibrosis and thickening of Glisson's capsule. Eventually the lobular pattern may be distorted by septums, justifying the diagnosis of cirrhosis [2732, 3317] that has been associated with disturbances of lobular blood flow [551].

Niemann-Pick Disease. In Niemann-Pick disease, phosphatides, primarily sphingomyelin, are found in excess in cells of the reticuloendothelial system and other histiocytic cells of almost every organ of the body, especially of the liver and spleen [3317]. The widespread nature of this condition, associated with severe malnutrition, mental retardation, and anemia, prevents survival beyond infancy. The disease is a rare, hereditary condition, predominantly found in young Jewish females.

The liver and spleen are equally involved, but in contrast to Gaucher's disease, hepatomegaly often precedes splenomegaly [1]. The Kupffer cells and periportal histiocytes are transformed to a characteristic foam cell, which has a central nucleus without giant-cell formation. Many highly refractile vacuoles in the cytoplasm stain with fat stains, and the cell is smaller than the Gaucher cell. Large nests of foam cells distort the architecture of the liver. Fibrosis is not part of the picture, apparently because the patients do not survive long enough for it to develop. The distortion of the architecture is not severe enough to cause changes in the results of hepatic tests [3317]. Lipid-containing lymphocytes and monocytes are found in the blood.

In gargoylism, also associated with hepatosplenomegaly, abnormal phospholipid deposition occurs, but mainly in bones.

Cholesterol-storage Disease. True cholesterol-storage disease, familial hypercholesteremia, does not involve the liver as a rule, even if associated with xanthomatosis. Infiltration of biliary ducts, portal tracts, and the hepatic parenchyma may occur in early infancy as part of a systemic lipid-storage disease, and the resulting extremely rare biliary cirrhosis has been called "fibroxanthomatous cirrhosis" by MacMahon. Secondary xanthomatosis as a result of liver disease, especially chronic cholestasis, has been discussed previously (see "Xanthomatous" Form of Cholangiolitic Cirrhosis, Chap. 46). Hand-Schüller-Christian disease

is a disorder characterized by granulomas, with deposition of cholesterol. Granulomas are found in the liver but are without functional significance. Systemic nonlipid reticuloendotheliosis, such as Letterer-Siwe disease or histiocytosis, is referred to elsewhere (see Reticuloendotheliosis, under Abnormal Hematologic Structures in the Liver, Chap. 60).

HEPATIC AMYLOIDOSIS

Amyloid consists of a protein moiety, possibly related to serum alpha globulin, and a carbohydrate moiety, chondroitin sulfate. In animals amyloidosis has been produced by hyperimmunization, viz., in horses used for diphtheria or antitoxin production, or by feeding large amounts of milk proteins. This corresponds with the finding that in China, where few dairy products are consumed, amyloidosis is rare [3107]. Amyloidosis apparently results from the formation and tissue deposition of abnormal serum globulins produced in hyperimmune reactions or by altered plasma cells. Amyloid deposition in the liver occurs in two forms, which differ in etiology and possibly in the chemical nature of the material deposited. In both instances the abnormal deposition starts around vessels, as if material oozing from the blood stream is imbibed by perivascular structures.

Pathogenesis. SECONDARY AMYLOIDOSIS. In systemic, or secondary, amyloidosis, amyloid is deposited around capillaries and to a lesser extent in the walls of arteries and veins of the parenchymal organs such as the liver, kidney, spleen, adrenal gland, and intestine. Although the spleen, kidney, and adrenal gland may be affected more often, the liver is involved in over 80 per cent of the cases [708]. Amyloidosis follows chronic disease associated with tissue destruction, particularly tuberculosis, chronic suppuration such as osteomyelitis, rheumatoid arthritis, neoplasms such as Hodgkin's disease or hypernephroid carcinoma, trichinosis, and lupus erythematosus [3309]. The amyloid in this condition combines readily with congo red and is strikingly metachromatic. It also gives a variable PAS reaction.

PRIMARY AMYLOIDOSIS. Primary amyloidosis has no apparent connections with inflammatory conditions and is found without apparent cause or in reactive or neoplastic plasmacytosis. It has mainly a mesodermal distribution, involving vessels and interstitial tissue, particularly in the myocardium, skeletal muscle, tongue, gingiva, cutis, and fat

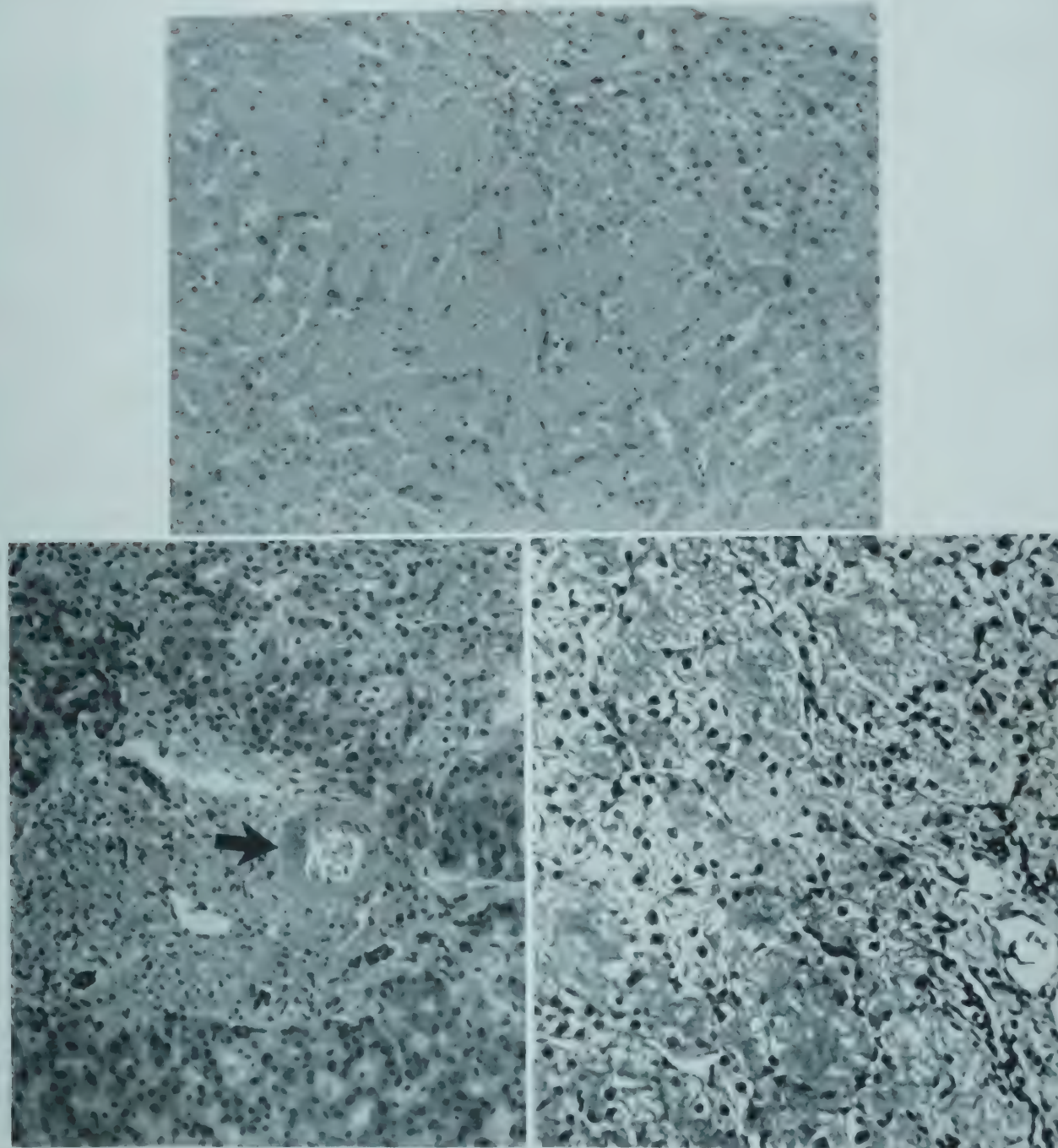


FIG. 177 Amyloidosis. *Upper*. Secondary amyloidosis with compression of hepatic cells. H&E ($\times 110$). (Popper, H., *Am.J.Med.* 16:98, 1954). *Lower left*. Amyloid inhibition of hepatic artery branch (arrow) in primary amyloidosis associated with myeloma. H&E ($\times 230$). *Lower right*. Amorphous amyloid compressing capillaries and hepatic-cell plates. H&E ($\times 230$).

tissue. Depositions in parenchymal organs are mainly related to vessels and not to the interstitial tissue. Sometimes one organ is predominantly involved, producing an amyloid tumor. The amyloid in primary amyloidosis does not stain so well with congo red, it is less metachromatic, has different solubilities [1412], and is possibly chemically different (para-amyloid). All forms of para-amyloid have been considered to result from circumscribed or diffuse plasma cell myelomas [85]. The protein moiety of amyloid is possibly a product of the

myeloma cells that has escaped from the blood stream. In myeloma with amyloidosis, no hyperglobulinemia is found. Hyperallergic amyloidosis has been thought to be the result of hyperglobulinosis owing to plasmacytosis. Many transitions have been observed between primary and secondary amyloidosis in distribution and etiology.

Clinical and Laboratory Findings in Secondary Amyloidosis. In secondary amyloidosis, liver involvement is indicated by hepatomegaly with or without splenomegaly. A positive congo red test

result after intravenous administration of the dye largely measures the hepatic amyloid deposition, since deposition in the liver is quantitatively more significant than in any other organ. Removal of more than 60 per cent of the injected congo red from the blood within 1 hour is suggestive of, and more than 90 per cent is diagnostic of, hepatic amyloidosis. Little functional impairment is noted in the results of the hepatic tests. If it is present it is not related to the extent of amyloid deposition. Occasionally increased Bromsulphalein retention is found [708, 3337]. The serum-albumin level is often low [3141, 3337], possibly because of the underlying disease. Cephalin flocculation and other protein reactions are normal [3141]. Elevation of serum-alkaline phosphatase activity has been reported [3141]. Slight elevations of the serum-bilirubin level occur, and occasional instances of jaundice have been reported [708, 3141]. While hepatic amyloidosis assists in the diagnosis of amyloidosis in general by the congo red test and by liver biopsy [708, 1525, 2992, 3350, 3440], it produces few manifestations. Recovery, with disappearance of amyloid, occurs with treatment of the primary disease. The clinical picture is dominated by the renal amyloidosis, which produces nephrosis and subsequently contracted kidney.

Structural Alterations in Secondary Amyloidosis. **MACROSCOPIC APPEARANCE.** The gross appearance of the liver depends on the extent of amyloid deposition. If it is severe, the liver is brown to gray, depending on the degree of amyloidosis, with a dry, waxy, transparent appearance on the surface and cut surface; the lobular architecture appears somewhat obscured because of relative anemia. The organ is enlarged, and its edges are blunted. Its consistency is firm and rubbery. Treatment with iodine produces a mahogany-brown color, which changes to blue upon treatment with dilute sulfuric acid.

HISTOLOGIC CHANGES. Amyloid deposition is found, mainly in the tissue spaces of the lobular

parenchyma between the hepatic-cell plates and capillaries (Fig. 177). Only in a minority of cases are the walls of the vessels involved. In these, deposition appears first between the muscle bundles of the media and subsequently in the adventitia. The veins are less commonly involved. Deposition may be seen only in circumscribed foci, or it may involve the greater part of the lobule, usually with peripheral or intermediate zone predominance. The central vein is rarely involved. The amyloid masses appear homogeneous and amorphous, but reticulum fibers traverse them. They compress the sinusoids, reducing the blood content of the liver. This also produces severe pressure atrophy of the hepatic-cell plates, with eventual disappearance of cells. In terminal stages, only masses of amyloid with compressed capillaries are noted. Connective tissue proliferation is not part of the picture.

Features of Primary Amyloidosis. Hepatic involvement in primary amyloidosis is almost as frequent as in the secondary form, occurring in 60 per cent of cases [1485]. However, in this report cases are included which show all the characteristics of secondary amyloidosis except for the absence of an etiologic factor. In several instances in which the morphologic description was that of secondary amyloidosis, jaundice was reported associated with increased thymol turbidity, serum alkaline phosphatase and serum cholesterol, Bromsulphalein retention, normal cephalin flocculation, and an increased prothrombin time [252, 708, 2493]. These findings have been explained as resulting from intrahepatic obstruction and loss of hepatic cells. In primary amyloidosis, defined by the staining qualities of amyloid and the primarily mesenchymal distribution, the arteries and periarterial tissue are the site of almost all the amyloid deposition (Fig. 177, lower left). This includes the intralobular arterioles, which become very conspicuous. Typical primary amyloidosis produces no gross changes in the liver and no functional hepatic alterations.

PART V

Focal Diseases of the Liver

Although focal hepatic diseases associated with granulomas are a heterogeneous group, including bacterial, mycotic, and parasitic diseases and disorders of unknown etiology such as sarcoidosis, they are presented together for differential diagnostic contrast. Granuloma-like lesions in lymphomas are described elsewhere (see Leukemias and Lymphomas, under Abnormal Hematologic Structures in the Liver, Chap. 60), as are eosinophilic granulomas (see Reticuloendotheliosis, under Relation between Liver and Hematopoietic System, Chap. 60). The focal nature of the disease processes does not cause functional changes, except when it is associated with diffuse non-specific reactive hepatitis or sometimes even with central necrosis. The presence of a granulomatous disease can often be diagnosed by liver biopsy, which sometimes even reveals the etiology of the granulomas.

HEPATIC TUBERCULOSIS

In tuberculosis, structural and functional alterations of the liver occur which result either from reaction to tuberculosis in other organs or from tuberculous granulation tissue within the liver. Tuberculous foci occur in three forms, all resulting from hematogenous dissemination.

1. Miliary granulomas or tubercles
2. Aggregated granulomas, or tuberculomas
3. Canalicular, or bile duct, tuberculosis

The latter two forms are rare and primarily of pathologic interest. Tubercles are common and are found in miliary tuberculosis and in organ tuberculosis. In both instances, the appearance and distribution of tuberculous granulomas are the same. More are present in miliary tuberculosis.

Miliary Tuberculosis

In miliary tuberculosis with or without pulmonary involvement, clinical evidence of hepatic injury is slight except for occasional hepatomegaly. Jaundice complicates or dominates the picture in rare instances [595, 2213]. In some instances, the miliary dissemination involves mainly the liver, spleen, and portal lymph nodes. In these forms liver biopsy is of particular advantage and may provide the only clue in undiagnosed febrile conditions, leading to prompt and life-saving specific antimicrobial therapy.

Laboratory Findings. Results of hepatic tests are erratic. Cephalin flocculation and thymol turbidity are often abnormal, and Bromsulphalein retention and serum-alkaline phosphatase activity are frequently slightly increased [1794, 2213].

Structural Alterations. **MACROSCOPIC APPEARANCE.** The liver is often enlarged and edematous. White nodules from 0.6 to 2.0 mm in diameter appear irregularly distributed throughout the parenchyma. Some are barely visible to the naked eye and are best seen through the capsule of the under surface of the left lobe.

HISTOLOGIC CHANGES. The use of liver biopsy has broadened the knowledge of the histologic appearance of the liver in miliary tuberculosis [676, 1364, 1632, 1794, 2213]. The observations on biopsy specimens agree with histologic examinations of necropsy specimens [1303, 2887]. The alterations noted in the liver in miliary tuberculosis can be divided into four groups:

1. Nonspecific reactive hepatitis with irregular hepatocellular damage, small areas of focal necrosis, diffuse Kupffer cell mobilization, and portal inflammation (Figs. 134, 179A). This hepatitis is

usually severe in miliary tuberculosis even in the absence of tubercles in the specimen.

2. Focal Kupffer cell proliferations, which occlude the lumens of the sinusoids, leading to compression and disappearance of hepatic cells between two sinusoids. Small nodules containing histiocytic elements (retothelial nodules) sometimes form [1364] (Fig. 178A, B, C, D).

3. Small foci of caseation necrosis, in which Kupffer cells and hepatic cells are necrotic, and which are surrounded by a few lymphoid and histiocytic elements [2992] (Fig. 178E).

4. Epithelioid tubercles, usually with Langhans' giant cells and with a variable rim of lymphoid and histiocytic elements (Figs. 178F, G, 179A).

Histiocytic nodules, foci of caseation, and tubercles are irregularly distributed throughout the lobule without reference to lobular topography and are therefore also found in the vicinity of the central vein (Fig. 178D, G). Fully developed larger tubercles are more often located near or within portal tracts (Fig. 179B, C). Tubercle bacilli are hardly ever demonstrated, either by Ziehl-Neelsen staining of the tissue section or by culture or guinea pig inoculation [2851].

MILIARY NECROSIS. In blood dyscrasias, such as leukemia, granulocytopenia, or pancytopenia, and in young infants with primary tuberculosis, the acute tuberculous dissemination produces large foci of necrosis almost devoid of giant cells, epithelioid cells, and lymphocytes. Sometimes hardly any cellular reaction is noted. In these foci, tubercle bacilli are easily demonstrated. The tuberculous dissemination is usually secondary to the blood dyscrasias. Only exceptionally does miliary tuberculosis cause acute leukemoid or pancytopenic reactions, which in turn alter the tissue response to tuberculosis.

Diagnostic Significance. In recent years the hepatic involvement in miliary tuberculosis has assumed clinical importance, because liver biopsy sometimes has led to this diagnosis in patients with unexplained fever and sometimes without roentgenologic findings in the lungs [595, 676, 1632, 1794, 2851, 2992, 3405]. In some patients pulmonary signs of miliary dissemination never became apparent, even after the liver biopsy, probably because the disease was then adequately controlled with vigorous antituberculous therapy.

Hepatic Reaction in Tuberculosis of Other Organs

In various types of organ tuberculosis, such as pulmonary, pleural, peritoneal, or osseous tubercu-

losis, hepatic alterations, including miliary tubercles, are found [1794, 2992]. Structurally, the tubercles represent miliary tuberculosis, but clinically the organ manifestations overshadow those of the dissemination. Liver biopsy often provides diagnostic clues as to the etiology of extrahepatic diseases in these instances.

Clinical and Laboratory Findings. Enlargement of the liver has been frequently reported in pulmonary tuberculosis and probably reflects fatty metamorphosis [1656]. Abnormal results in almost all hepatic tests have been found in a somewhat erratic fashion in pulmonary and extrapulmonary tuberculosis [1581, 1794, 1870, 2147, 2992]. Abnormal results of cephalin-flocculation and thymol-turbidity tests are caused by increased gamma globulin [2989] and other serum protein changes [3375]. Serum mucoproteins and fibrinogen are increased, and occasionally serum-alkaline phosphatase activity is increased. Bromsulphalein retention is frequently abnormal [1581, 1870], and the total cholesterol is low and supposedly more closely mirrors the clinical findings than any other test [2992].

Structural Alterations. Nonspecific reactive hepatitis is the most frequently found lesion [1364, 1794, 2907, 2992, 3405] and probably accounts for the impairment of hepatic function and for some of the gamma globulin elevation [2907]. This is followed in incidence by histiocytic nodules [1364] and by tubercles. In autopsy specimens in which large sections are examined, tubercles have been found in 40 to 80 per cent of the cases [1303, 2887]. In the small biopsy specimens, they are not frequent and their incidence depends upon the size of the specimen [3313, 3405]. They are found in pulmonary and extrapulmonary tuberculosis, and the idea that intestinal tuberculosis is required for infection of the liver by the portal route has been abandoned. Conglomerate or fibrosed tubercles are seen in protracted tuberculosis and in healing stages (Fig. 179D).

CIRRHOSIS AND TUBERCULOSIS. A stellate shape or scarring of the portal tracts is noted in protracted organ tuberculosis. Sometimes this progresses to perilobular fibrosis. Occasionally, part of a lobe appears separated by septums, resulting in small regenerative nodules. This supports the opinion that cirrhosis may result from tuberculosis [1303, 2797]. Cirrhosis develops in guinea pigs experimentally infected with tuberculosis, but its occurrence in man has not been convincingly demonstrated [1696]. The frequent coincidence of cir-

B

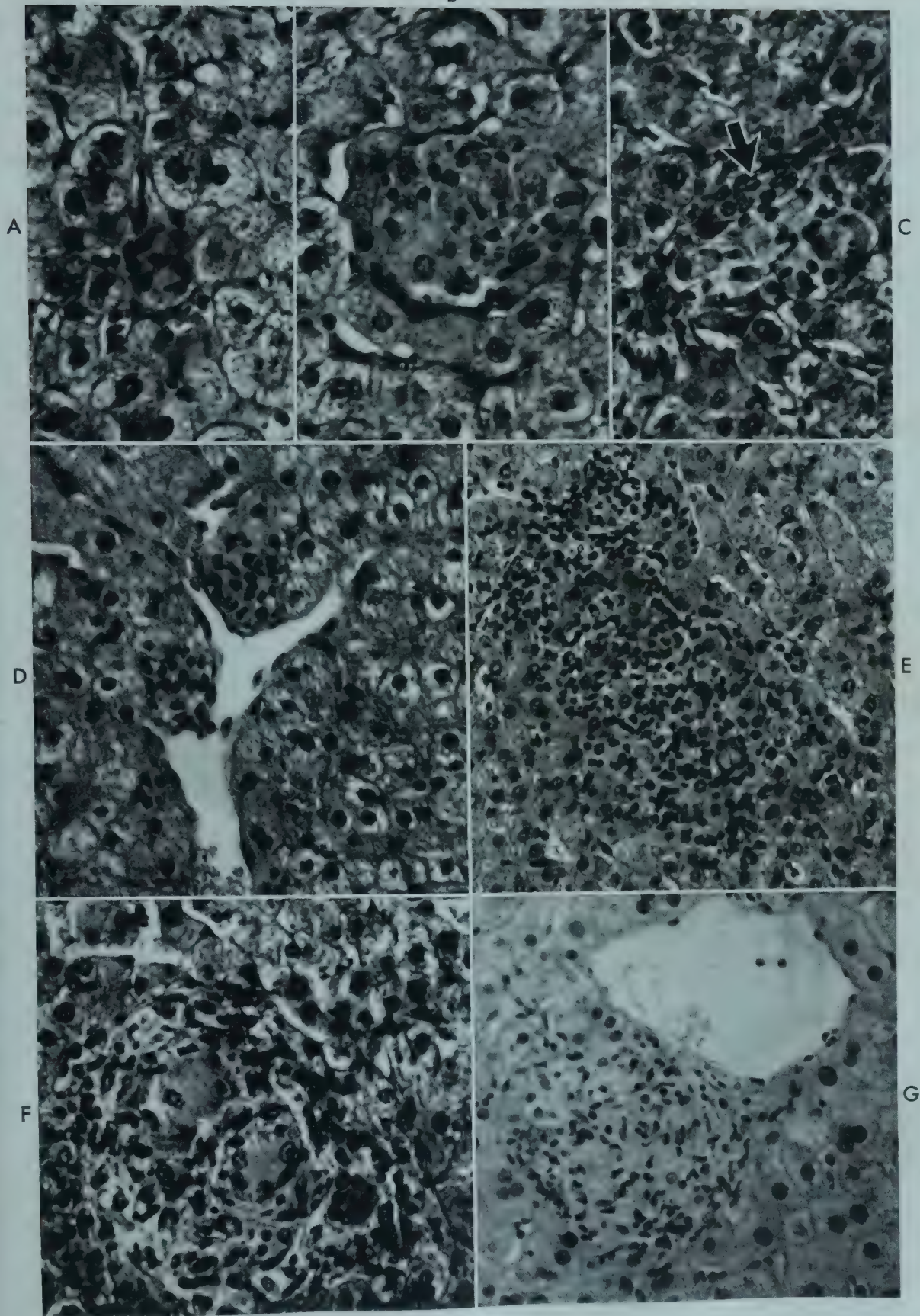


FIG. 178 Biopsy specimens of active tuberculosis. H&E. A. Hepatic-cell damage, Kupffer cell mobilization, and small accumulation of histiocytes ($\times 315$). B. Same as in A, showing larger histiocytic accumulation, with some cells becoming epithelioid ($\times 315$). C. Histiocytic nodule adjacent to intralobular ductule (arrow) ($\times 315$). D. Histiocytic nodule below the intima of the central vein ($\times 230$). E. Intralobular focal necrosis and caseation surrounded by histiocytes ($\times 180$). F. Recent epithelioid cell tubercle with giant cells near portal tract ($\times 230$). G. Epithelioid tubercle near central vein ($\times 230$).

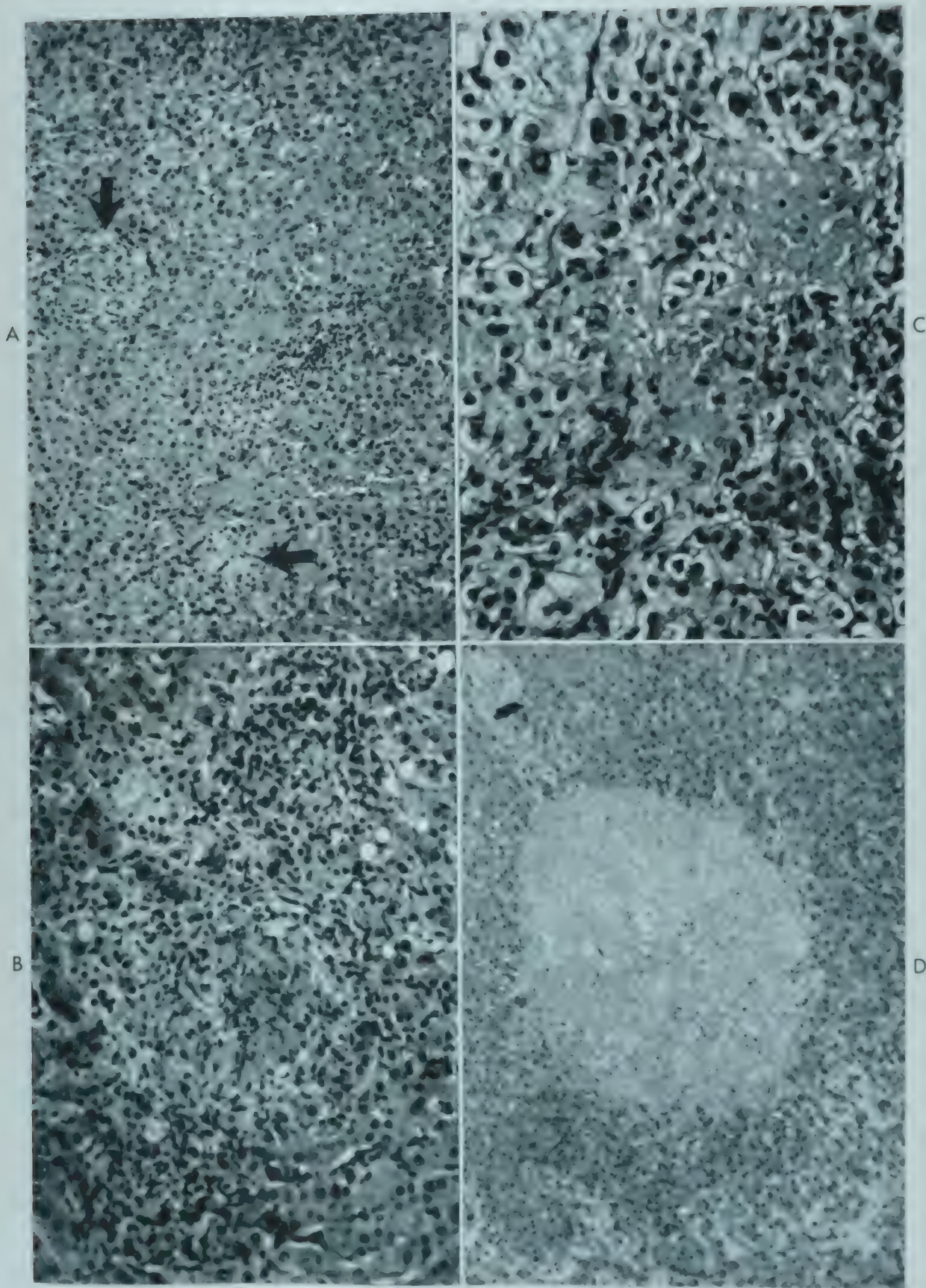


FIG. 179 Tuberculosis. H&E. A. Miliary tuberculosis with several epithelioid cell tubercles (arrows), focal necrosis, Kupffer cell mobilization, and portal inflammation ($\times 105$). B. Central caseation in tubercle near portal tract ($\times 140$). C. Extensive caseation in large periportal tubercle ($\times 210$). D. Fibrosed tubercle ($\times 57$).

rhosis and tuberculosis in man that has been noted in liver biopsy specimens [2992] is explained by concomitant nutritional disturbances, including alcoholism.

FATTY METAMORPHOSIS. Fatty liver was common in tuberculosis [2887, 2992], but now it is limited almost entirely to intestinal and peritoneal tuberculosis [1656] and is seldom seen in biopsy specimens, except if alcoholism is present. Improved dietary management is responsible for the disappearance of fatty metamorphosis.

Focal dilatation of the capillaries, or peliosis, almost angiomatic in character and associated with blood cysts, occasionally occurs in chronic tuberculosis (see Peliosis, under Alterations of the Sinusoids, Chap. 56).

Structural Characteristics of the Hepatic Tubercle

Development of Hepatic Tubercle. The full-grown tubercle seems to develop through two pathways:

1. Histiocytic elements, either hepatic or blood-borne, accumulate around small necrotic or caseous foci, in response to the preexisting tissue injury (Fig. 178E). Segmented leukocytes and lymphocytes also participate. In this stage, viable hepatic cells in caseous foci surrounded by histiocytic elements are not easily differentiated from epithelioid cells. Subsequently, the histiocytic elements become transformed into epithelioid cells, and their cytoplasm swells and becomes acidophilic. Fibroblasts, especially near portal tracts, may also become epithelioid cells. Eventually some epithelioid cells develop into Langhans' giant cells, supposedly by amitotic nuclear division without partition of the cytoplasm [1303]. Transformation of sinusoidal endothelial cells and even of bile duct epithelial cells into such giant cells can also be seen.

2. Tubercles develop without preceding hepatocellular necrosis by accumulation of histiocytic cells, which are eventually transformed into epithelial cells (Fig. 178B).

Lymphoid elements appear very early around the tubercle, forming a shell (Fig. 178F). Tubercles near the portal tracts produce inflammatory changes in the tracts, sometimes with ductular proliferation (Fig. 179C). In the center of larger tubercles particularly, secondary necrosis of the epithelioid cells occurs, producing a core of a fine granular acidophilic tissue intermixed with a few fine basophilic nuclear fragments (caseation necrosis) (Fig. 179C). In reticulum fiber stains, the fibers may appear broken in small areas of casea-

tion but less frequently in secondary caseation, because the developing epithelioid cells have pushed aside the original fiber framework. In less acute forms of miliary tuberculosis, especially those under treatment, and in organ tuberculosis, fibrosis and conglomeration of tubercles develop.

DEVELOPMENT OF FIBROSIS. Fibrosing changes are characterized by three features, depending upon the connective tissue alteration in the acute stage of the granulomatous lesion.

1. If the reticulum fiber network is not broken and caseation is not extensive, collagenous membranes form within the tubercle and become thicker and sclerosed, scarred collagenous tissue eventually replacing the original nodule. Such small scars persist for a long time.

2. After extensive caseation of tubercles, especially conglomerate tubercles, collagenous membranes are deposited as shells, preceded by the appearance of fibroblasts on their periphery. These shells are demonstrated with Van Gieson stains and may surround caseated material indefinitely. Calcification occurs, and nodules up to 0.5 cm in diameter are found as innocent scattered residuals.

3. Around tubercles, collagenous membranes extend into the surrounding parenchyma and lead to "sclerosis" of the liver. This is most prominent around portal tracts, producing portal and sometimes perlobular fibrosis. This fibrosis occasionally develops without tubercles [1696, 2887].

A local effect of the tubercle bacilli or their products apparently accounts for both primary and secondary caseation. Kupffer cell mobilization and histiocytic nodules which resemble the nodules in typhoid fever or Hodgkin's disease [1364] probably produce local ischemia by obstruction of sinusoids, thus contributing to the necrosis.

Histologic Differential Diagnosis. Tubercles can be recognized with the naked eye in biopsy specimens [3313], but they may not necessarily be found in single sections. Multiple or serial sections must be performed to exclude the presence of tubercles, especially if nodules are seen grossly. The demonstration of a tubercle is evidence only of a granulomatous disease. A few other criteria are of assistance in pointing to tuberculosis in preference to other conditions:

1. The demonstration of acid-fast bacilli, either in the section or by culture, is the only absolute evidence, although this is rarely possible.

2. Localization of tubercles within the lobular parenchyma independently of the portal tracts,

especially in the vicinity of the central vein, seldom occurs in conditions other than tuberculosis, and almost never in sarcoidosis.

3. The pattern of caseation also assists somewhat in differentiation from sarcoidosis (see Sarcoidosis, later in this chapter) but not from other granulomatous diseases. Usually the etiology of the hepatic granuloma seen in liver biopsy specimens has to be established by collateral methods.

Hepatic Tuberculomas

Tubercles occasionally coalesce to form large lesions, which may be the size of an apple. One or several of such tuberculomas may be found in the liver, giving the impression of a tumor or of an abscess [1303, 1919]. Tuberculomas are more common in children and in Negroes. This is a rare condition, clinically associated with fever, chills, and hepatomegaly, almost always accompanied by tuberculosis in other organs, especially the vertebra [1919], but not with intestinal tuberculosis [1303]. Large caseated foci are also found in the very rare instances of congenital tuberculosis transmitted through the placenta with a primary focus in the liver and parallel involvement of the portal nodes.

Canalicular Tuberculosis

Sometimes conglomerate tubercles involve the bile ducts, the bile of which discolors the caseated areas. Either a tubercle develops in the bile or the duct is invaded from the outside. Multiple green caseated nodules are rare [1303, 2822]. The portal lymph nodes are extensively caseated as a rule. Acute or subacute tuberculous cholecystitis is also rare and is usually associated with gallstones [1919, 3403].

Relation between Tuberculosis and Hepatic Injury

Tuberculosis as such produces little functional hepatic change. The structural lesions are of importance from a diagnostic standpoint but not as an indication of disturbed hepatic function. Little evidence exists for tuberculous hepatitis or cirrhosis. However, reactivation of tuberculosis elsewhere in the body aggravates an existing hepatitis and facilitates the transition of fatty liver into cirrhosis (see Transition of Fatty Liver into Cirrhosis, Chap. 51). On the other hand, hepatic injury such as acute hepatitis or cirrhosis seems to aggravate tuberculosis. Tuberculous peritonitis develops as a complication of cirrhosis, and re-

activation of tuberculous foci is commonly found at autopsy in progressing cirrhosis. Protein changes by tuberculosis are temporarily altered by hepatitis [2559], possibly with changes in antibody formation.

HEPATIC SARCOIDOSIS

The knowledge of the clinical and pathologic manifestations of visceral sarcoidosis has been advanced by liver biopsy [1091, 1147, 2057, 2757]. The etiology of the disease is not known. It has been considered a peculiar reaction to tubercle bacilli, a lesion caused by other specific microorganisms, or a nonspecific response to various types of injuries [2057]. The individual lesion, the sarcoid follicle, and its natural history have been extensively studied [170, 1091]. The sarcoid follicle shows no caseation, peculiar cytoplasmic inclusion bodies, predominantly cellular reaction, and proliferation of reticulum fibers instead of destruction as seen in the tubercle caused by tubercle bacilli [2057, 2848]. Differentiation of the sarcoid follicle from the tuberculous granuloma is morphologically difficult, and patients with typical sarcoidosis seem readily to contract tuberculosis [936, 2057]. Similarities between sarcoidosis and hyperergic reactions, as well as granulomatous reactions to beryllium, asbestos, and silica [936], have been noted.

Hepatic Reaction in Sarcoidosis

The liver may be enlarged in sarcoidosis. Some investigators report this in approximately 20 per cent of all cases [1147]; in cases with follicles proved by biopsy, enlargement is noted with greater frequency [1794, 3027].

Laboratory Findings. The hepatic tests reflecting the elevation of gamma globulin level typically found in the disease [1091, 2057, 2757, 3027] show abnormal results, including abnormal cephalin flocculation and thymol turbidity. Albumin is often decreased [1794, 3027], and electrophoretically albumin and alpha globulin levels are low, being lower in active cases than in inactive ones [1019, 1794]. Serum-alkaline phosphatase activity is increased in approximately 30 per cent of cases [1794, 3027]. This elevation does not parallel that of serum bilirubin and is not related to bone lesions or hypercalcemia [1388, 3027]. Bromsulphalein retention occurs even less frequently, and hyperbilirubinemia occurs only ex-

ceptionally in the absence of hepatic disease [1794, 3027].

Incidence of Hepatic Sarcoidosis in Biopsy Specimens. Hepatic follicles were reported in approximately 40 per cent of cases at autopsy [170, 1091, 2057, 2757, 2848]. A recent review records an incidence of 66 per cent [378], including a significant number of cases in which sarcoidosis was found incidentally and was undergoing healing.

The incidence of positive findings reported in the small biopsy specimen is higher than in the large autopsy tissue section, because biopsies are performed mainly in florid sarcoidosis. After the original, almost incidental, observation [3404], a series of studies reported [1794, 2901, 3027, 3406] an increasing incidence of hepatic sarcoidosis. In a recent review [378] 70 per cent positive results in 63 cases of sarcoidosis were recorded, and in our own observations 90 per cent positive results were found in the first liver biopsy performed in a large group of patients [3313].

The diagnostic reliability of liver biopsy in active sarcoidosis is enhanced by use of a biopsy needle which provides a large enough specimen and by gross inspection of the specimen, with subsequent study of serial sections, especially if no follicles are found in routine histologic sections although the gross appearance suggested their presence [3313].

Structural Alterations. Follicles in other organs all show the same stage of development [2057, 2757] and seem to persist for long periods of time without any alterations. This results in a uniform appearance of the follicles throughout the body. In contrast, the size and stage of the follicles in the liver vary greatly [1794, 3027, 3313], suggesting a more rapid maturation and regression. This and the associated nonspecific reactive hepatitis in active sarcoidosis produce a polymorphous picture in the liver.

MATURE FOLLICLE. The characteristic mature follicle of sarcoidosis is larger than a high-power field, being up to 2 mm in diameter (Fig. 180, upper left and right). It consists of epithelioid cells arranged in a haphazard fashion and intermixed with some large and irregular giant cells. They rarely show inclusion bodies, and Schaumann's asteroid bodies do not occur, in contrast to the situation in lymph nodes. The follicle is surrounded by a rim of histiocytes and lymphocytes, a few of which are found within the follicle. The

reticulum framework within the follicle differs from the surrounding tissue and is usually denser, indicating new formation of fibers. The central zone is sometimes structureless, smudgy, very acidophilic and refringent, i.e., it shows fibrinoid degeneration. Where two follicles touch each other, a laminated protein material is deposited, considered to be para-amyloid and derived from excess serum gamma globulin [3309] (Fig. 180, upper right). The follicle is best discerned with Masson trichrome stain [1794]. The follicles vary in size. With the use of large specimens and serial sections in each instance, some granulomas are found which contain more than 12 epithelioid cells on the cut section, and at least one contains multinucleated giant cells. The follicles are usually contiguous with the portal tracts [1794, 2901, 3027], but follicles are also seen within the parenchyma [3313]. Serial sections indicate that such follicles are related either to portal tracts or to intralobular ductules, and almost invariably proliferation of ductules is noted about follicles (Fig. 180, lower left). The ductular proliferation is possibly related to the increased serum-alkaline phosphatase activity. Histochemically, phosphatase is increased in the vascular endothelium about the follicles [3027]. In addition to follicles, nonspecific reactive hepatitis is usually seen, with Kupffer cell mobilization, focal necrosis, portal inflammation, and accumulation of histiocytic cells, as well as focal fatty metamorphosis.

LIFE CYCLE OF THE FOLLICLE. The development of the follicle in the liver, as studied by biopsy [3027, 3313], is similar to the known cycle in other organs [170, 2057]. The initial lesion seems to be a proliferation of a few histiocytic cells, presumably Kupffer cells, usually associated with necrosis and disappearance of hepatic cells. The larger lesion consists of a small nest of histiocytic cells, many of which can be proved related to periportal or to intralobular ductules (Fig. 180, lower left). Several cell accumulations are usually arranged in grapelike fashion. Whether the initial nodule develops near lymphatic vessels or ductules, and whether it is mature if it is in contact with either structure are unknown. Further maturation of the follicle entails transformation of the histiocytes into epithelioid cells and giant cells and the shell-like accumulation of lymphocytes. Follicles aggregate mainly around the portal tracts and are enveloped by para-amyloid, which persists after the aggregation (Fig. 180, upper right).

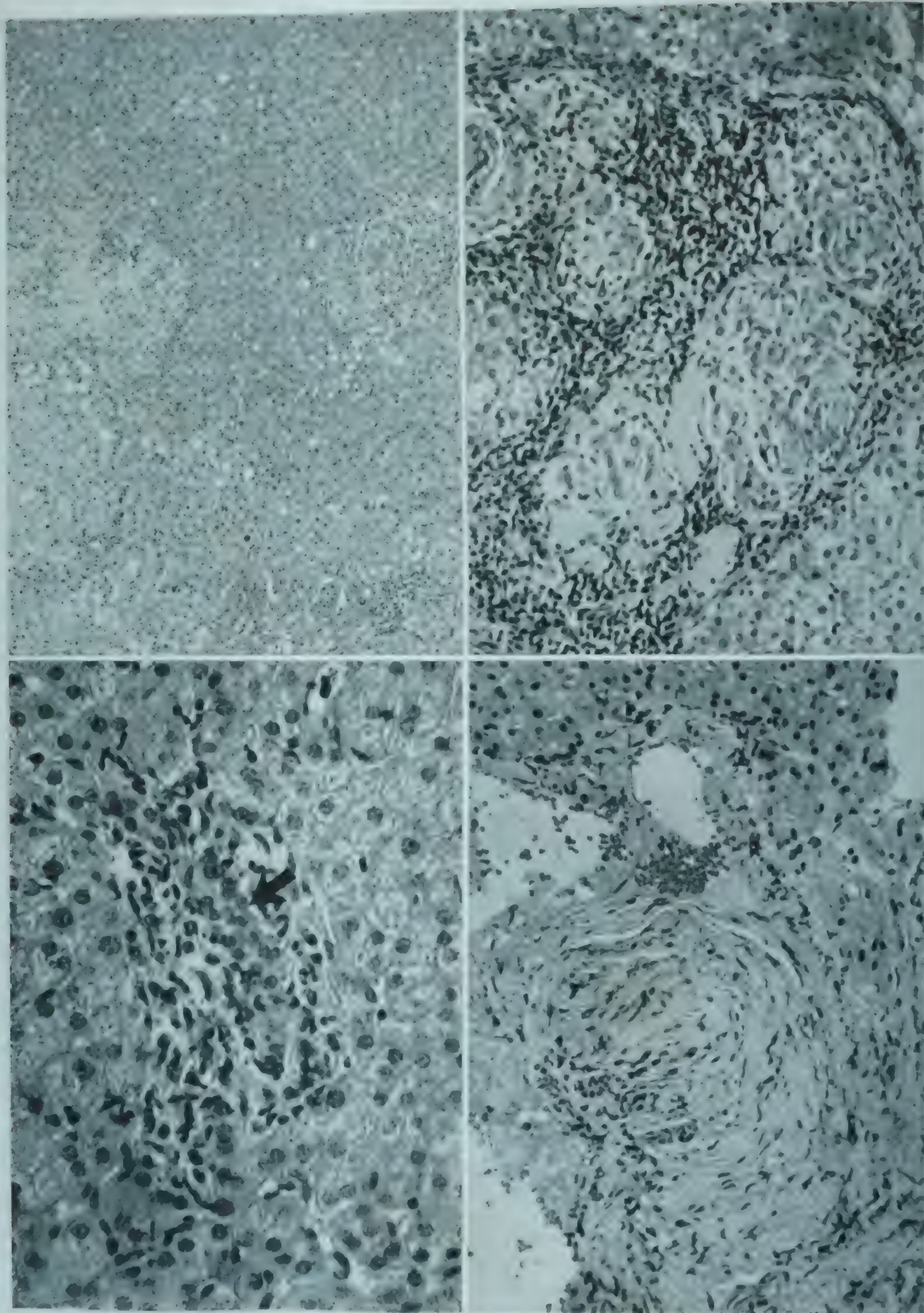


FIG. 180 Sarcoidosis in biopsy specimens. H&E. *Upper left* Fully developed sarcoidosis with several conglomerated follicles containing giant cells and exhibiting "hyalinization" of the stroma (para-amyloidosis). The intervening parenchyma shows some focal necrosis ($\times 55$). *Upper right* Conglomerated follicles composed of epithelioid cells and a few giant cells surrounded by lymphocytes and homogenous connective tissue (para-amyloidosis) ($\times 120$). *Lower left* Histiocytes and epithelioid cells around intralobular ductule (arrow) ($\times 230$). *Lower right* Fibrosing follicle ($\times 120$).

Collagenous fibers are laid down around single or conglomerate follicles. Regression is characterized by development of central coagulation necrosis, which seldom becomes extensive. In addition to the para-amyloid and collagenous envelope, fibrosis occurs in the follicle, and the giant cells disappear, the epithelioid cells shrink and possibly develop into fibroblasts. Reticulum fibers and subsequently concentrically arranged collagen fibers and membranes are laid down, and eventually a body of whorled fibers is formed (Fig. 180, lower left). These fibrotic and fairly characteristic scars do not seem to persist; in autopsy specimens, scars are found in other organs, e.g., lung, lymph nodes, and heart, but not in the liver.

Histologic Differential Diagnosis. Absolute criteria establishing the diagnosis of sarcoidosis from liver biopsy specimens are not available, and in most instances confirmation is required by clinical and other laboratory methods. Such confirmation depends mainly on the exclusion of other diseases, since the etiology of sarcoidosis is not established. The differential diagnosis of sarcoidosis, which is usually benign, from tuberculosis is especially important because steroid hormone therapy, used in sarcoidosis, is contraindicated in tuberculosis. The following features assist in excluding diseases other than sarcoidosis:

1. A follicle exceeding a high-power field in size and associated with giant cells speaks against brucellosis and other diseases with extensive focal necrosis.
2. Localization of the follicles and their restriction to the vicinity of portal tracts and intralobular ductules, as seen in serial sections, speaks against tuberculosis, while the presence of granulomas near the central field is a stronger argument for excluding sarcoidosis.
3. Caseation is more apparent in tuberculosis, although this is relative. The fibrinoid necrosis in sarcoidosis stains best with Mallory's phosphotungstic acid hematoxylin and is supposedly more refringent and acidophilic than tuberculous caseation.
4. The limitation of the nodule is sharper in sarcoidosis than in tuberculosis, and the nodule is surrounded by a shell of para-amyloid. In late stages, a whorled appearance of the fibrous scar suggests sarcoidosis.

Sarcoidosis as a Hepatic Disease

Relatively rarely does sarcoid involvement of the liver cause sufficient clinical manifestations to

cause liver disease to become the presenting disorder and even lead to death. This fact explains the varying manifestations. For instance, "nodular periportal" cirrhosis with sarcoid granulomas has been seen in a patient dying from tuberculosis [2057]. Portal hypertension with splenomegaly, which was surgically relieved, has also been found [862]. Intrahepatic cholestasis associated with splenomegaly, prolonged jaundice, and greatly increased serum-alkaline phosphatase activity, with varying degrees of hepatocellular degeneration, occurred in a few patients with sarcoidosis, confirmed at autopsy [378] or by biopsy [1091, 2835, 3456]. In all these instances, extensive sclerosing granulomas of the liver, which seem to have led to at least beginning septum formation, were found. Moreover, hemolytic jaundice without hepatic involvement occurs in sarcoidosis [427, 3172].

Sarcoidlike Lesions

Erythema Nodosum. The nosologic position of this condition is not known, but it is considered to be a hypersensitivity reaction to various agents, of which tuberculosis may be one. In some of the few cases in which liver biopsies have been performed, granulomatous nodules have been described which resemble sarcoidosis or tuberculosis [1794].

Berylliosis. In beryllium intoxications, nodules of the same type as in sarcoidosis are found in various organs, including the liver [562].

Eosinophilic Infiltrations. More commonly in children than in adults heavy infiltration of the portal tracts with eosinophilic leukocytes occurs. This infiltration sometimes takes the form of granulomas [3732]. Clinically, hepatomegaly is associated with circulating eosinophilia. Some of these mainly transient reactions are possibly the result of parasitic infestations.

Nonspecific Granulomatous Hepatitis. Hepatic granulomas are occasionally encountered without any apparent cause [1794]. Many are similar to those seen in sarcoidosis (Fig. 181, upper left). Sometimes they are associated with chronic pulmonary granulomatosis [766].

HEPATIC BRUCELOSIS

Brucellosis is a generalized infection by the very invasive *Brucella suis* or *melitensis* or the less invasive *Brucella abortus*. In each condition, hepatic granulomas occur frequently, but only in

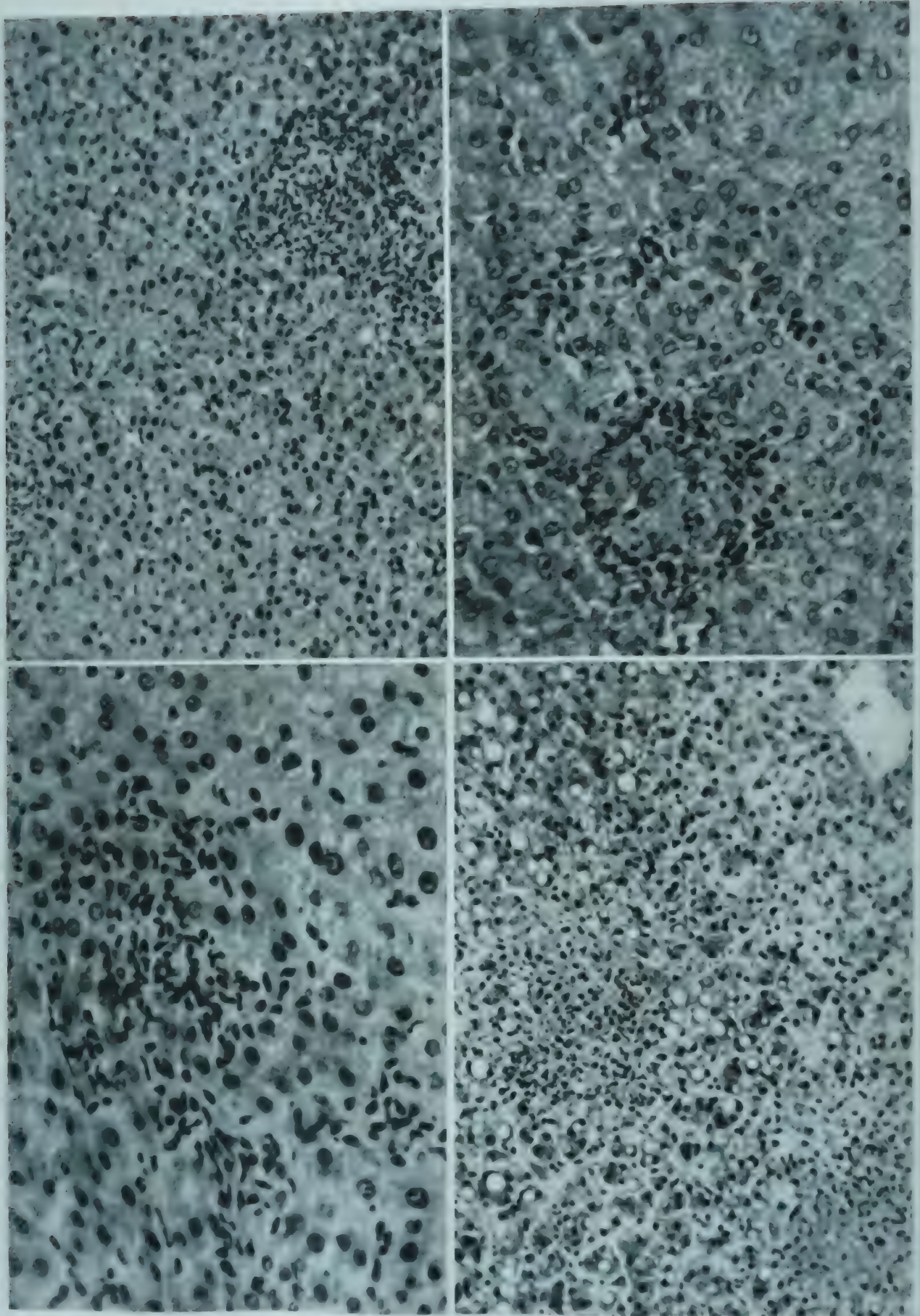


FIG. 181. *Upper left.* Granulomas morphologically similar to tubercles of unknown etiology. H&E ($\times 240$). *Upper right.* Hepatitis from brucellosis. Multiple intralobular and periportal granulomas composed of epithelioid cells, histiocytes, and segmented leukocytes can be seen, as well as diffuse hepatic-cell damage and focal necrosis. H&E ($\times 130$). *Lower left.* Circumscribed granuloma in pa-

relatively few instances does a hepatic disease result. Nevertheless the hepatic granulomas assist in the demonstration of the activity of the process.

Hepatic Reaction in Brucellosis

Hepatic enlargement is not a frequent finding in brucellosis, and no consistent abnormalities are noted in the hepatic test results [3158]. Nevertheless, the liver biopsy specimen usually shows a dramatic picture [168, 1679]. *Brucella* have been cultured from biopsy specimens [2112] and from bile.

Structural Alterations. In agreement with well-controlled experimental studies [419] and autopsy studies [30], biopsy specimens in brucellosis caused by *B. abortus* show a diffuse, nonspecific reactive hepatitis, with Kupffer cell mobilization and portal infiltration with various inflammatory cells, necrosis, and granulomas (Fig. 181, upper right). The smallest lesion is an aggregation of histiocytic cells and of some proliferated Kupffer cells around necrotic hepatic cells [1794]. In addition, larger granulomas are found, either within the lobules or contiguous to the portal tracts (Fig. 181, lower left). These granulomas consist of epithelioid cells with occasional Langhans' giant cells and coagulation necrosis in the center of the granuloma. The granulomas contain segmented neutrophils and are separated from the surrounding tissue by an indistinct lymphocytic rim. The epithelioid cells are more loosely arranged than in a typical tuberculous granuloma. In addition, large areas of focal necrosis are found, surrounded by a rim of lymphocytes, plasma cells, histiocytes, and a sprinkling of segmented leukocytes (Fig. 181, lower right). Fibroblastic reaction with formation of collagenous fibers and membranes develops in and around nodules with central necrosis. In *B. melitensis* or *B. suis* infections, large areas of necrosis predominate [92].

The absence of clinical and functional manifestations associated with impressive structural alterations [1679, 3158] has been partly clarified by serial biopsies, as well as by experimental studies. In the initial acute stage, clinical and functional manifestations, including fever [1679], are associated with predominance of epithelioid cells

and granulomas without necrosis, while in afebrile reinfections of the liver, necrosis and fibrosing nodules are found. In guinea pigs [92, 419], the initial experimental infection leads to proliferation of Kupffer cells and histiocytes containing the microorganisms, while reinfection, possibly in old foci, produces necrosis. The polymorphous picture frequently seen in liver biopsy specimens therefore suggests repeated hepatic invasions. The changes are probably influenced by variation in hypersensitivity or resistance of the host or in the virulence and number of microorganisms.

Differential Diagnosis. Liver biopsy does not permit an unqualified diagnosis of brucellosis but only the diagnosis of a granulomatous lesion. The etiology has to be established by clinical and especially by microbiologic means. The following features suggest brucellosis:

1. A polymorphous appearance with simultaneous presence of histiocytic nodules, epithelioid and giant-cell tubercles, and areas of necrosis, the latter two varying in size in the same specimen. Polymorphism is usually not seen in tuberculosis and sarcoidosis. The occurrence of predominantly small lesions, of less than a single high-power field in diameter, speaks for brucellosis.
2. Large areas of necrosis surrounded by relatively little inflammatory exudate. This does not occur in sarcoidosis and is rare in tuberculosis. Such foci may occur in tularemia.
3. The presence of segmented neutrophils. This is fairly frequent in the granuloma of tularemia and brucellosis but seldom occurs in tuberculosis or sarcoidosis.
4. Loose arrangement of epithelioid cells in the granulomas [1679].

Hepatic Disease from Brucellosis

An acute hepatitis phase occurs in brucellosis [1679], with hepatomegaly, jaundice, and even ascites and with abnormal results of the hepatic tests, especially of cephalin flocculation and albumin/globulin ratio [603]. Portal inflammation and periportal formation of membranes develop in the acute stage. Cirrhosis following brucellosis has been reported more often in Europe [1679, 2044, 3158] than in America [3695]. At least one case

tients with brucellosis, composed of histiocytes and a few epithelioid cells. Nearby are areas of focal necrosis with segmented leukocytes. H&E ($\times 230$). Lower right. Extensive focal necrosis with segmented leukocytes in a fatty liver in an autopsy specimen from a patient with brucellosis. H&E ($\times 120$). (Courtesy of Dr. J. Arias-Stellas.)

is on record [2112] in which follow-up biopsies showed the development of septal cirrhosis from brucella hepatitis. A further possibility, suggested in a specimen studied [92], is the development of florid cirrhosis caused by brucella infection in a fatty liver (Fig. 181, lower right).

HEPATIC SYPHILIS

The tremendous progress in diagnosis and antibiotic therapy has greatly reduced the incidence of syphilitic lesions in the liver. Furthermore, most cases of hepatitis occurring during antisyphilitic treatment are the result of serum hepatitis and are not related to the syphilitic infection (see Antisyphilitic Treatment Jaundice, under Epidemiology, Chap. 42). Finally, many cases which were thought to be syphilitic cirrhosis because of a positive serologic reaction are now known to be the result of other etiologic factors, especially nutritional ones. In recent years, few reports concerning hepatic syphilis have appeared [1340, 3022, 3286], but it is extensively discussed in most textbooks. Hepatic syphilis assumes the following forms: (1) gummatous hepatitis, a rare clinical condition but important as a differential diagnostic possibility [3022]; (2) *hepar lobatum*, mainly an incidental pathologic finding; (3) the rare granulomatous, or diffuse, syphilitic hepatitis, difficult to separate from other forms of hepatitis [3158]; (4) congenital syphilis, which has become extremely rare in the United States. Reference is made to other texts for detailed discussion [943, 1996, 2804].

Congenital Syphilis

In infants with congenital syphilis, confirmed by positive serologic findings in the mother, either gummas or diffuse hepatitis may occur. Both are associated with manifestations in other organs, but jaundice is infrequent, even with severe involvement of the liver. The gummas are more often found in the left lobe of the liver and are circumscribed areas of necrosis surrounded by occasional giant cells and often related to the portal tract (Fig. 182A). In diffuse interstitial hepatitis, the liver is firm, green, and slightly enlarged. Histologically, in addition to small miliary gummas and frequent hematopoietic foci, a diffuse proliferation of collagenous fibers and membranes distorts the hepatic-cell plates and separates single cells or small groups of cells (Fig. 182B). These cells may

show excessive regeneration, with syncytial giant-cell formation. The fine fibrillar connective tissue is heavily infiltrated with lymphocytes and also with segmented leukocytes. Fibrosis is especially severe around the vessels and below the capsule, producing a gummatous pericholangitis and pylephlebitis near the hilus of the organ. Spirochetes are abundant in untreated cases in the gummas, as well as in the interstitial tissue. Many instances that previously would have been diagnosed as syphilitic interstitial hepatitis of infants are actually neonatal giant-cell hepatitis, probably of viral etiology (see Giant-cell Hepatitis, Chap. 46). Congenital syphilis after the neonatal period produces findings similar to those in the acquired form [943, 2804].

Diffuse Syphilitic Hepatitis in Adults

Hepatic Involvement in Secondary Syphilis. Many references can be found in earlier texts to the existence of hepatic lesions and jaundice in early secondary syphilis, apparently simultaneously with skin manifestations, "*icterus syphiliticus praecox*" [2804]. This condition has become extremely rare, and, in view of the lack of experience, one can only speculate as to the presence of a nonspecific reactive hepatitis. A female predominance has been claimed, which suggests the possibility of other etiologic factors such as abortifacients. Viral hepatitis and infectious mononucleosis can not be excluded. Even fatal hepatitis (acute yellow atrophy) has been reported [1470]. Some of the cases occurred after arsenical treatment and were therefore considered to be the result of antisyphilitic therapy, rather than of syphilis itself (see Antisyphilitic Treatment Jaundice, under Epidemiology, Chap. 42). The high incidence of jaundice in syphilitic patients under treatment with arsenic led to the claim that arsenic therapy is contraindicated in jaundice. However, improvement of jaundice in syphilitic hepatitis with arsenotherapy has also been observed [1949].

Diffuse Hepatitis in Tertiary Syphilis. In patients with untreated syphilis, prolonged deep jaundice develops in rare instances, associated with a firm large liver, splenomegaly, and a strongly positive serologic reaction for syphilis. No other etiologic factor is evident. Functionally, hepatocellular degeneration and cholestasis are noted. Histologically, a diffuse hepatitis with a moderate degree of hepatic-cell damage, extensive Kupfer-cell mobilization, focal necrosis, and even small

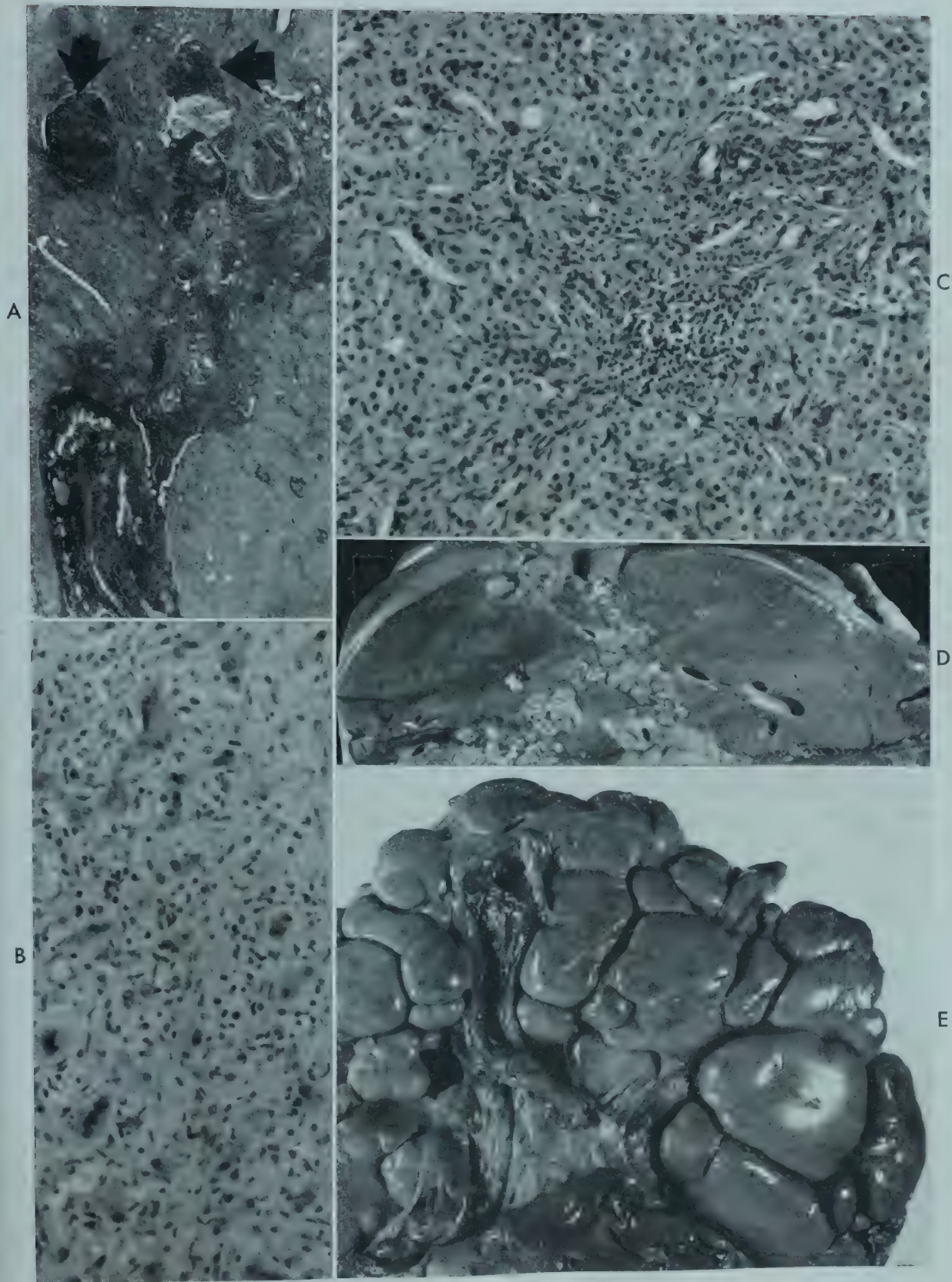


FIG. 182 Syphilis of the liver. A. Gumma in liver of child with congenital syphilis. Large vascular scars and circumscribed necrotic foci (arrows). Mallory's aniline blue ($\times 3\frac{1}{2}$). B. Congenital interstitial hepatitis in an infant. The hepatic-cell plates are separated by fibrous tissue. Some of the hepatic cells are multinucleated. H&E ($\times 115$). C. Nonspecific hepatitis with hepatomegaly in tertiary syphilis which subsequently improved on antisiphilic therapy. Portal infiltration, ductular proliferation, focal necrosis, and moderate hepatic-cell damage can be seen. H&E ($\times 115$). (Courtesy of Dr. E. R. Hayes.) D. Multiple hepatic gummas. E. Hepar lobatum.

granulomas with portal infiltration and ductular proliferation are seen (Fig. 182C). If after anti-syphilitic treatment, especially with iodides or penicillin, the liver shrinks, jaundice subsides and the results of hepatic tests improve, one may be justified in assuming a syphilitic etiology.

Hepatic Gummas and Hepar Lobatum

The syphilitic lesions of the liver most frequently encountered now are scars which deform the organ to varying degrees and in which active gummas are sometimes found, i.e., "hepar lobatum." This lesion is mainly diagnosed at autopsy but occasionally produces peculiar palpatory findings and, even less frequently, functional and clinical alterations. In recent years, at least in the clinical and pathological material observed by the authors, it has not presented a clinical problem. Apparently the extensive and successful treatment of the basic disease has left relatively few instances of hepar lobatum, and these are mainly pathologic curiosities.

Clinical Manifestations. The classic descriptions of several decades ago report fever, jaundice, and a very much enlarged and irregular liver [943, 2804]. The differential diagnosis of the lesion included cirrhosis, chronic peritonitis and perihepatitis, amyloid disease, hepatic tumors, abscesses, gallstones, chronic splenic anemias, or Banti's syndrome, and biliary cirrhosis [2804]. About 16 per cent of the syphilitic patients seem to have had hepatic involvement.

The descriptions of recent years differ from the earlier descriptions [1340, 3022, 3286]. Fever is not a significant feature, the liver is seldom lumpy, and the spleen is not enlarged. Jaundice, apparently resulting from mechanical involvement of the bile ducts by the scars, is found in approximately one-sixth of the cases, ascites in almost one-third, and portal hypertension with esophageal varices leading to hematemesis in about one-fourth of the cases. Spider nevi are absent.

In one series almost one-third of the patients studied died as the result of hepatic syphilis, and the majority of these deaths resulted from esophageal hemorrhage [3022]. The results of serologic tests for syphilis were abnormal in more than 80 per cent of the cases studied, whereas in the remainder a preceding history or rapid response of the liver to antisyphilitic treatment suggested the diagnosis of syphilis.

Structural Alterations. The liver is one of the

most common sites of development of gummas, the acute granuloma produced by syphilis [3286]. The necrotic gumma nodules may coalesce to produce large lobular nodes, up to several centimeters in diameter, which are prominent over the liver surface. They may be solitary or multiple, and they are usually irregularly demarcated (Fig. 182D). The recent gumma is gray-red and rather soft. In the necrotic area the preexisting framework is preserved and can be demonstrated by fiber stains. The necrosis is partly the result of obstructive inflammation of the intima of the blood vessels. On the edge of the gumma, granulation tissue with relatively few epithelioid cells and occasionally with giant cells is found, and scarring starts relatively early (Fig. 182A). In previous years emphasis was laid on the facts that in the gumma, in contrast to the tuberculoma, more fibroblasts, lymphocytes, and plasma cells and fewer epithelioid cells are found, and that caseation necrosis is more diffuse but that the original structure can still be demonstrated. The gumma can be partially or completely absorbed, leaving large irregular scars, which are characterized by many vessels and extension from the surface deep into the liver. These scars, together with a reactive fibrous perihepatitis, account for the deformity of the hepar lobatum (Fig. 182E). Severe scarring in the right lobe was claimed to cause compensatory hypertrophy of the left lobe, and pathologic museums exhibit unusually deformed livers. Such lesions were often associated with penile ulcers, bone lesions, aortic aneurysms, and, apparently less frequently, with syphilitic central nervous system manifestations. Gummas may also be associated with amyloidosis.

HEPATIC GRANULOMAS IN VARIOUS DISEASES

Granuloma-like lesions have been noted in typhus [41], typhoid and paratyphoid fever, glanders, and bubonic plague [1303]. Some of these lesions were actually miliary abscesses that resembled granulomas.

Tularemia. In tularemia, large areas of focal necrosis are encountered, up to 2 mm in size and yellow in color [250, 292, 1061]. In the center of the area of coagulation, necrosis, nuclear debris, and scattered segmented leukocytes are found. The boundaries of these areas are not sharp, and on their periphery a few segmented leukocytes

lymphocytes, and giant cells are noted. The associated clinical manifestations usually serve to differentiate them from brucellosis or tuberculosis.

Leprosy. In lepromatous leprosy, with generalized visceral involvement late in the disease [2523], granulomas frequently occur in the liver; these lesions are called "miliary lepromas" [109, 3339]. They have been found in liver biopsy specimens [1579]. The granulomas consist of histiocytic cells which appear as epithelioid cells or as the foamy leprosy cells rich in acid-fast bacilli. They are mainly found near the portal tracts. In addition, small histiocytic nodules are observed throughout the parenchyma. Isolated Kupffer cells are enlarged and contain many bacilli. The abundance of bacteria serves for the differentiation from tuberculosis. Miliary lepromas undergo fibrosis and rarely give rise to cirrhosis.

Blood Dyscrasias. In agnogenic myeloid metaplasia [364, 2608] and panmyelopoiesis, as well as in Hodgkin's disease [3285], granulomatous lesions occur in the liver which are discussed in detail elsewhere (see Leukemias and Lymphomas, under Abnormal Hematologic Structures in the Liver, Chap. 60).

FUNGUS INFECTIONS OF THE LIVER

Histoplasmosis

The fungus *Histoplasma capsulatum* produces primarily an infection of the reticuloendothelial system. It was originally considered a severe, ordinarily fatal disease and was usually associated with hepatic involvement and splenomegaly [2527]. At present it is known to be frequently a mild disease, with only transient active changes, mainly in the lung, and probably exceptional hepatic involvement. Functional impairment with abnormal results of flocculation tests occurs only with diffuse involvement, especially with cirrhosis formation. Anatomically, three types of lesions are noted in the liver, some of which can be seen in biopsy specimens [581].

GRANULOMA FORMATION. The most common lesion seems to be a small tubercle-like granuloma consisting of irregularly arranged epithelioid cells and some giant cells, partially surrounded by lymphocytes (Fig. 183A). Central necrosis is frequently seen, and in the surrounding parenchyma nonspecific reactive hepatitis is found. Giant cells or Kupffer cells in the surrounding parenchyma

may contain the fungus, but this is not common; when it occurs the differentiation from sarcoidosis [2734] or tuberculosis is difficult morphologically.

KUPFFER CELL INVASION. The Kupffer cells and, occasionally, wandering histiocytes in the portal tracts are invaded by *Histoplasma*, especially in autopsy specimens. The organism is an oval body, 2 to 5 μ in diameter, best demonstrated by PAS staining of its capsule (Fig. 183D). The enlargement of Kupffer cells sometimes results in multinucleated giant-cell formation. The lesion resembles kala-azar (Fig. 183E), in which no capsule can be demonstrated.

DIFFUSE SEPTAL CIRRHOSIS. This is the rarest lesion and is caused either by coalescence of granulomas (Fig. 183B) or by disturbed sinusoidal blood flow by the enlarged Kupffer cells (Fig. 183C), with resulting central rather than portal septum formation.

Actinomycosis

The ray fungus, *Actinomyces bovis*, often invades the liver in actinomycosis [3530] by various routes [654]:

1. Direct invasion from other organs occurs, owing to the characteristic disregard of actinomyces infections for anatomic barriers. The fungus enters the liver from pleural or pulmonary infections through the diaphragm, or it spreads from the liver into the pleura, producing a hepatopleural-pulmonary communication (Fig. 184A). Appendiceal or colonic actinomycosis also extends to the liver.

2. Invasion through the portal vein, usually originating from the appendix, leads to multiple abscesses [654, 985].

3. Rare hematogenous infection occurs through the hepatic artery, producing disseminated abscesses. Occasionally the primary site has subsided, and then the liver lesion represents the predominant finding.

CLINICAL MANIFESTATIONS. Clinical findings of hepatic actinomycosis depend on its spread and extent. When the abscesses are large, hepatomegaly, abdominal pain with right upper quadrant tenderness, and sometimes a mass are present. Leukocytosis is commonly found, but jaundice is rare, and the results of the hepatic tests are usually not abnormal.

The diagnosis is possible only by the demonstration of the ray fungus in pus or in a biopsy specimen from the liver or from one of the sinuses (Fig.

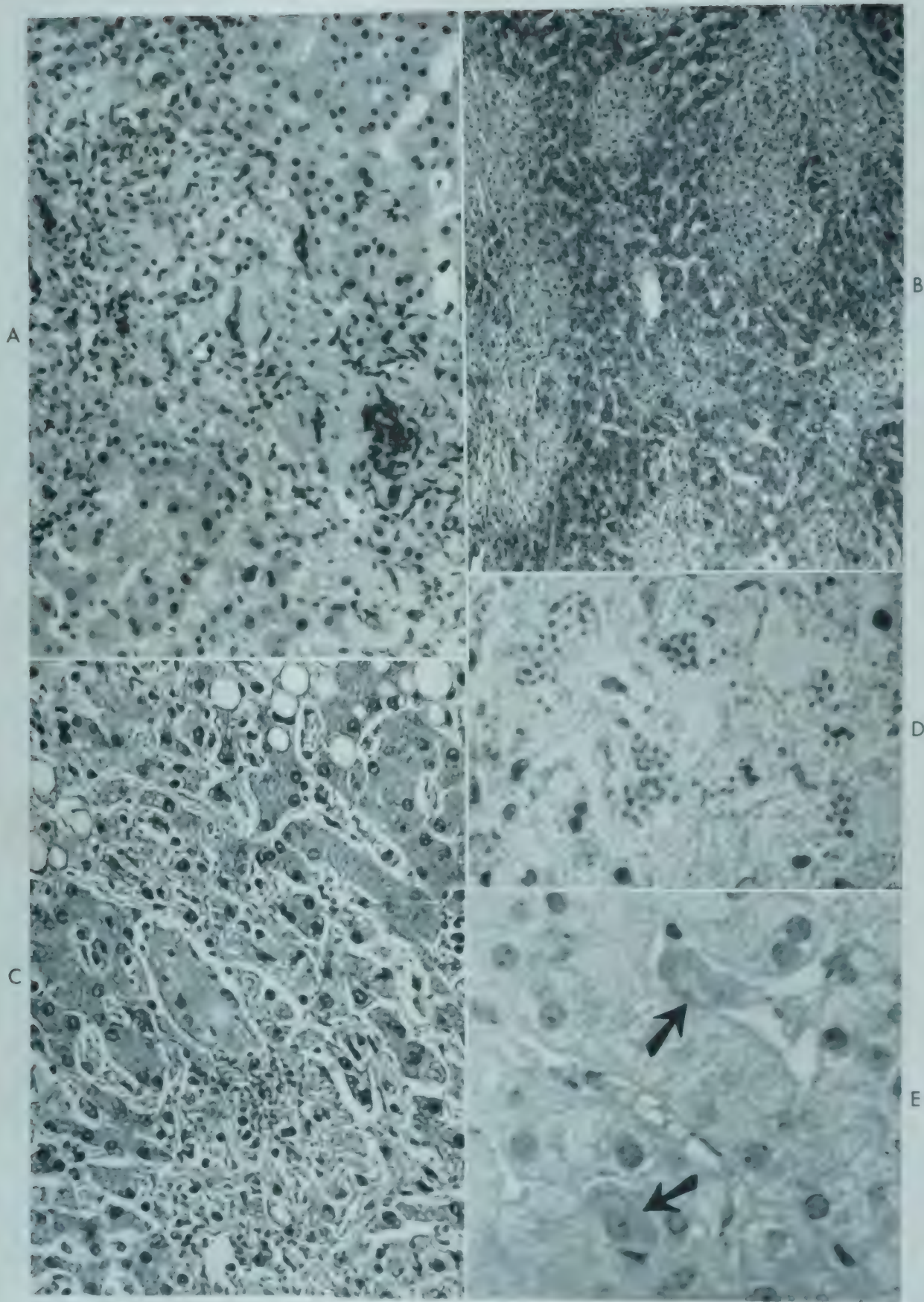


FIG. 183 A. Liver biopsy specimen in histoplasmosis proved by culture from liver. An epithelioid cell tubercle with many giant cells of various shapes can be noted, partially surrounded by lymphocytes. H&E ($\times 190$). B. Same liver as in A, showing multiple granulomas, which in places extend from the portal tracts and obscure the lobular architecture. H&E ($\times 60$). C. Sinusoids obstructed by Kupfer cells, which are greatly enlarged and multinucleated, in autopsy specimen of a patient with cirrhosis. H&E ($\times 220$). D. *Histoplasma capsulatum* in Kupfer cells. Giemsa ($\times 455$). E. Kala-azar showing Leishman-Donovan bodies (arrows) in enlarged Kupfer cells. Giemsa ($\times 455$).

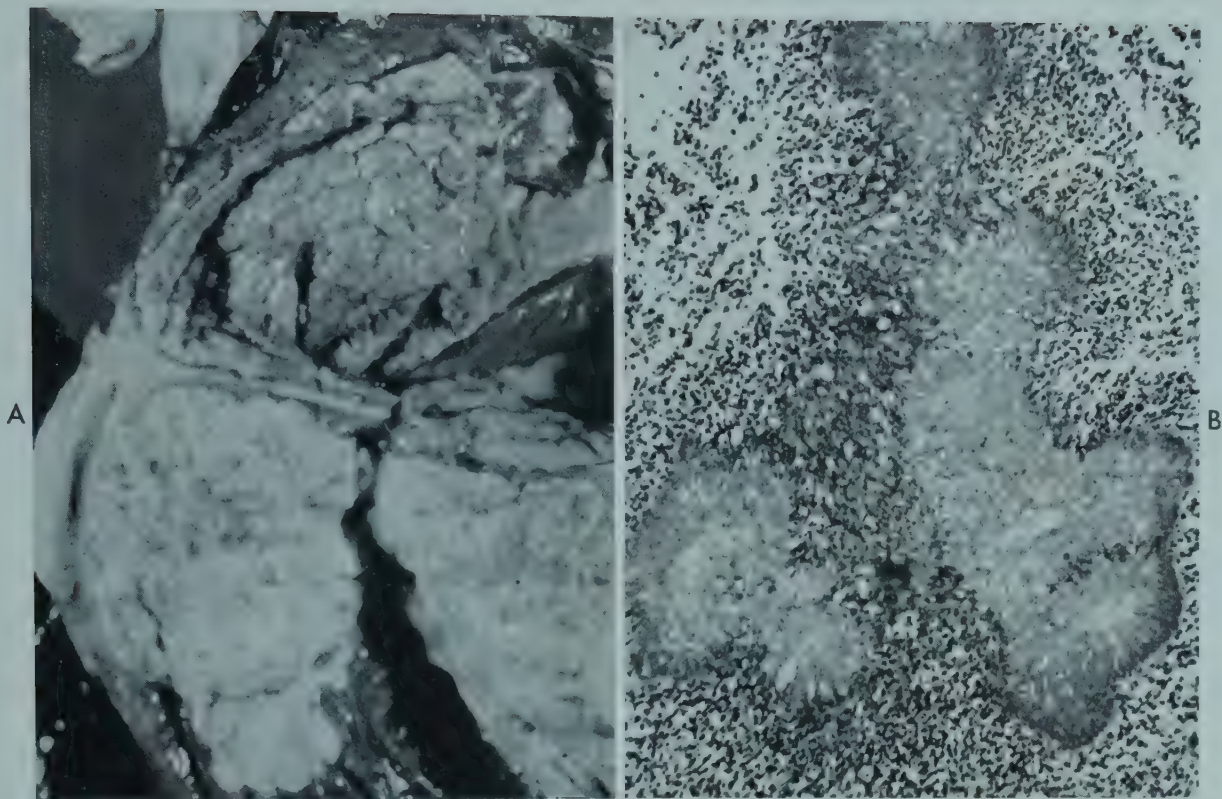


FIG. 184 A. Liver abscess caused by actinomycosis communicating with empyema cavity, which in turn communicates with pulmonary abscesses. (Wangensteen, O. H., Snapper, I., and Popper, H.: *Rev. Gastroenterol.* 19:950, 1952.) B. *Actinomyces bovis* in liver abscess. H&E ($\times 125$).

184B). In biopsies sometimes only nonspecific epithelioid cell granulomas are found [1794].

STRUCTURAL ALTERATIONS. *A. bovis* infection is characterized by the formation of abscesses which readily coalesce, producing a honeycomb appearance of the lesion. In the center of the abscess the ray fungus lies in a necrotic area, in turn surrounded by granulation tissue in which segmented leukocytes and many lipophages are found. The lipophages are responsible for the gross yellow color of the lesion. The surrounding liver tissue shows compression, as well as fibrosis of the portal tracts with ductular proliferation. Large abscesses form by coalescence with daughter abscesses in the vicinity or with abscesses further away. Multiple abscesses develop, especially if the portal vein is involved. The hepatic abscess may perforate into neighboring structures, such as the pleura and lung (Fig. 184A), or through the skin, producing honeycomb fistulas. Involvement of the gallbladder occurs exceptionally. The prognosis of hepatic actinomycosis is usually grave. Treatment includes use of various medications, such as sulfonamides, iodides, and antibiotics, and radiation or surgery.

Other types of *Actinomyces*, such as the aerobic *Nocardia asteroides*, may produce granulomas in the liver.

Other Fungus Infections

Hepatic granulomas occur in almost any systemic fungus infection and have been reported in blastomycosis [435] and coccidiomycosis [3225]. A hepatosplenoglandular form of blastomycosis has been described in South America.

DIFFERENTIAL DIAGNOSIS OF HEPATIC GRANULOMAS

Liver biopsy is now widely used for the recognition of granulomatous diseases in the hope of establishing the etiology of unclear febrile conditions. The importance of the size of the specimen for the demonstration of the granuloma and the use of special stains, including Masson's trichrome stain, have been discussed (see Applications of Liver Biopsy, Chap. 39). Nevertheless the differential diagnosis of the hepatic granuloma itself remains a difficult problem. It depends on three criteria.

1. Demonstration of the offender in the biopsy specimen itself, either in the granuloma or in the surrounding liver tissue. This can be done generally in blastomycosis, coccidiomycosis, actinomycosis, and leprosy; less frequently in histoplasmosis

and tularemia; and only exceptionally in tuberculosis.

Many parasites produce granulomatous lesions in the liver, especially *Strongyloides*, *Ascaris*, *Schistosoma*, *Fasciola*, and *Toxocara* [3199]. Small discrete lesions resemble tubercles composed of epithelioid and giant cells, sometimes intermixed with eosinophils, and arranged around a necrotic zone, or ova (Fig. 185, left). Larger lesions show a granulation tissue and extensive necrosis and severe eosinophilia. Parasites can usually be found, at least in serial sections.

2. Collateral clinical and laboratory evidence of the primary disease. This is possible in syphilis, tuberculosis, berylliosis, brucellosis, blood dyscrasias, and fungus and parasitic diseases. Diseases with unestablished etiologies, such as sarcoidosis

and erythema nodosum, present problems.

3. The histologic appearance of the granuloma itself. The tubercle with central necrosis surrounded by epithelioid and giant cells and demarcated by lymphocytes occurs in tuberculosis, sarcoidosis, berylliosis, histoplasmosis, brucellosis, schistosomiasis, syphilis, and leprosy. In typical instances, certain characteristics of the tubercle are helpful, but in an individual lesion in a liver biopsy specimen, almost all criteria may fail. The situation is further confused by the occurrence of similar tubercles without any recognizable disease. The use of liver biopsy has proved valuable, since the demonstration of granulomas in the liver is a major diagnostic guide. Increasing experience of the pathologist may reduce the incidence of indecision.

Protozoa, helminths, and a few insects invade the liver. The textbooks of parasitology and tropical diseases discuss the subject more fully than this chapter, which emphasizes primarily structural and functional alterations of focal lesions. Parasites producing diffuse hepatic lesions such as those of malaria and kala-azar have been described under hepatitis (see Malarial Hepatitis, Chap. 45).

PROTOZOAN INFESTATIONS OF THE LIVER

Hepatic Amebiasis

Endamoeba histolytica is a protozoan parasite frequently found in the human intestine. As a result of a disturbance in the parasite-host relationship the liver may be invaded via the mesenteric and portal veins.

Involvement of the liver in amebiasis occurs in 36 per cent of cases in patients dying with amebiasis and in 8 per cent of cases of clinical amebiasis [3524]. The incidence is gradually decreasing. Hepatic amebiasis occurs mainly in males and in the middle-age groups. The interval between intestinal and hepatic involvement varies from several days to several years.

LIFE CYCLE. The motile form, or trophozoite, of *E. histolytica* is 10 to 30 μ in size. In warm stool specimens a single large pseudopod is seen to extend in the direction of progression. The cytoplasm contains many vacuoles and, frequently, ingested red cells, in contrast to that of the nonpathogenic *Endamoeba coli*. The trophozoites rapidly die outside the body, while smaller nonmotile cysts, 5 to 20 μ in diameter, which represent the infective form, survive rather drastic treatment. Four nuclei are characteristically found in each

cyst. The cysts are ingested with contaminated food and pass unchanged through the stomach, but in the small intestine the cyst wall is digested. Four amebas are hatched from each cyst. The amebas attach themselves to the intestinal mucosa and migrate into the crypts. They penetrate into the submucosa by secreting a cytolytic substance. A small cavity is produced by the amebas, which utilize red cells and tissue remnants for food. The absence of a leukocytic response militates against the term "abscess." Amebas and cysts may be discharged into the intestinal lumen to maintain the life cycle of the parasite. Clinical symptoms referable to the intestine are not necessarily present. This carrier stage is widespread throughout the world and accounts for the endemic nature of the disease in some areas, including the United States.

A disturbance in host-parasite relations produces colitis or even dysentery, as well as hepatic, pulmonary, or cerebral involvement with or without colitis. The hepatic involvement may assume many forms, some of which are not clearly understood.

Hepatomegaly during Amebic Enteritis. Persons with intestinal amebiasis frequently have enlargement of the liver. Autopsy studies in such persons fail to reveal the presence of amebas [502]. The rapid response of this enlargement to therapy with nonabsorbable intestinal amebicides suggests that nonspecific reactive hepatitis is responsible for the hepatomegaly [754, 3128]. Earlier necropsy studies revealed focal necrosis, focal fatty metamorphosis, and portal inflammation similar to changes in other forms of dysentery [2518].

Acute Amebic Hepatitis. Amebic hepatitis seems to be a diffuse disease, the nature of which is not established and which only recently aroused greater interest, although "presuppurative" hepa-

titis had been mentioned years ago. Some investigators consider local necrotizing and reactive regenerative processes produced by the amebas the cause of amebic hepatitis and therefore different from the "abscess" only in extent and distribution [2472]. Others deny any characteristic pathologic features [1708].

CLINICAL MANIFESTATIONS. Acute amebic hepatitis is characterized by an enlarged tender liver, dirty fading sun-tan pigmentation of the skin, right upper quadrant pain, fever, and anorexia [1785, 3696]. Intestinal involvement is not necessarily demonstrated [1785, 3696], and on careful examination amebas have been found in 66 per cent of cases [1996]. Jaundice is uncommon. In subacute amebic hepatitis, low-grade and intermittent fever are present, while in the chronic form liver pain is present for many months in the absence of fever [1785].

LABORATORY FINDINGS. Moderate leukocytosis is observed, and complement-fixation test results are frequently positive [3696], while the results of the hepatic tests are usually normal [1450, 1785, 3063, 3129, 3696]. Bromsulphalein retention or cephalin flocculation is occasionally abnormal. The low incidence of abnormalities in this condition, in contrast to those in viral hepatitis, is striking and of diagnostic importance. The response to specific antiamebic therapy is dramatic [1785, 3063, 3696], and whatever liver function impairment is present quickly disappears [489].

STRUCTURAL ALTERATIONS. Amebas enter the portal tributary and reach the liver probably by the portal route rather than through the lymphatic vessels or by direct extension. Invasion of the liver is probably common, but the amebistatic ability of the liver often stops the amebas [986]. Nevertheless they may produce thrombotic obstruction of small portal radicles, which facilitates their cytolytic action upon surrounding tissue. This minute lesion, or microabscess, sometimes progresses, forming a large abscess. In other instances it lingers on or heals, with or without scarring [299]. The final outcome depends on several factors, such as the extent of parasitic invasion, the host resistance, bacterial superinfection, alcohol intake, trauma, and therapy [1463].

The assumption that amebic hepatitis is a stage of a diffuse but attenuated invasion is supported by animal experiments. In kittens, a lesion similar to that in man, characterized by thrombotic occlusion of small vessels, has been produced by intestinal infection [501]. In hamsters, hepatic necrosis

has been produced by direct inoculation of amebas into the liver [2737]. The concept that amebic hepatitis is a *forme fruste* of abscesses, or microabscesses, is not supported by liver biopsies, in which only nonspecific reactive hepatitis was found in patients with protracted fever [345, 1450, 2518]. Further investigations, especially with liver biopsies, are required to establish whether amebic hepatitis is a nonspecific reaction, a hypersensitivity process, or microabscesses.

Amebic "Abscess" of the Liver. Amebic "abscess" is a very severe disturbance of the host-parasite relationship. One of the factors responsible for this condition seems to be exposure of an individual from the Temperate Zone to tropical conditions. This explains the much higher incidence in the tropics and the use of the term "tropical abscess." Natives in the tropics are usually spared.

Although the term is in common use and in some areas "tropical liver abscess" is the most frequent hepatic "abscess," it is a misnomer. The connotation "tropical" should not be considered an indication that the lesion is not found in the United States.

Amebic abscess is not associated with accumulation of pus and disintegration of segmented leukocytes but is a cytolytic necrosis of the liver. The amebas themselves, however, are not found in the necrotic area but are in the still viable tissue, surrounded by little reaction. This has given rise to the theory that the cytolytic factors are released only by the dying parasites [1620].

CLINICAL MANIFESTATIONS. In the acute stage, anorexia, weight loss, chills and fever, nausea, and pain occur, usually associated with cough, owing to diaphragmatic involvement, and enlargement of the liver, especially of the right lobe, with circumscribed tenderness upon compression. Roentgenologically, the liver is enlarged, and the right hemidiaphragm is often fixed [754, 1785, 3696]. Jaundice is somewhat more common than in amebic hepatitis [754]. The absence of diarrhea even in the history does not exclude the lesion. As the process becomes older, fever and pain subside, while weakness and loss of weight become more prominent. Even without complications, the mortality rate is 7.0 per cent, being particularly high with multiple abscesses [754].

LABORATORY FINDINGS. Leukocytosis is frequent. The complement-fixation test for amebiasis is recommended for confirmation of the diagnosis. Amebas are found in the stool with varying de-

grees of frequency, ranging up to 47 per cent in well-studied groups [754]. Abnormal results in any of the hepatic tests are the exception rather than the rule. Serum-alkaline phosphatase activity and Bromsulphalein retention are sometimes increased [390]. Needle puncture of the tropical abscess is historically the oldest diagnostic needle aspiration of the liver and long antedated needle biopsy. Demonstration of a sterile abscess by puncture may unmask a chronic amebiasis and at the same time is a useful therapeutic procedure [3415].

STRUCTURAL ALTERATIONS. Many small abscesses may be present in both lobes [1620]. They are circumscribed areas of necrosis, yellow-white in color and not clearly defined. As they become larger, they usually coalesce, and a central cavity develops. The main abscess is usually single and is generally located in the right lobe near the dome, with fibrous or fibrinous adhesions to the diaphragm, or on the inferior surface near the hepatic flexure of the colon [754]. In the center of the abscess cavity a chocolate-colored, thick fluid is found as a result of seepage of blood from digested vessels in the walls of the cavity. The membrane lining the cavity is shaggy, thin, yellow, and irregular and is composed largely of debris. A necrotic and necrobiotic layer of liver tissue and a layer of inflammatory reaction and fibrosis surround the cavity. The amebas are found on the border between the necrotic and necrobiotic zones. Round-cell infiltration in the portal tracts, bile duct proliferation, and focal and periportal necrosis are also seen. The inflammatory reaction may eventually be associated with the formation of regenerative nodules and of membranes condensing to septums, which subdivide the lobule in circumscribed areas. Consequently the border zone of an older amebic abscess often presents the picture of a septal cirrhosis. Amebas are never found in this inflammatory reactive zone, and the stimulus for this reaction appears to be the breakdown products of liver tissue in the necrotic zone.

Complications of Hepatic Abscess. All complications are serious and have a mortality rate of 43 per cent [754].

BACTERIAL INFECTION. Infection of the abscess cavity results in transformation of the necrobiotic cavity into a true abscess filled with pus. The wall assumes the character of a pyogenic membrane; clinically, increased fever, chills, and leukocytosis develop.

INVOLVEMENT OF THE DIAPHRAGM. This leads to pleurisy [505] or empyema and eventually to in-

volvement of the lungs, so that a combined hepatopulmonary abscess results. The abscess drains into a bronchus, and a hepatobronchial fistula is established [754]. The location of the abscess in the dome of the liver facilitates this process, and various factors such as the virulence of the infection and resistance of the host decide its fate.

RUPTURE AND METASTASES. Rupture of a hepatic abscess into the peritoneal cavity causes peritonitis, which may localize and form subphrenic or subhepatic abscesses. Hematogenous dissemination to the lung produces pulmonary abscesses. These abscesses in the lung may also rupture through the diaphragm back into the liver. Distant metastases to such sites as the brain may also occur.

Therapy. Hepatic amebiasis is characterized by a dramatic response to antiamebic therapy. Three therapeutic approaches to hepatic amebiasis are available.

The first is systemic, directed toward the eradication of the organism in hepatic tissues. The treatment of choice is considered to be chloroquine, 1.0 gm per day for 2 days, followed by 0.5 gm daily for a total of 3 weeks [384, 635, 931, 3696]. This is usually combined with oxytetracycline, 500 mg every 6 hours for 10 days. Emetine hydrochloride, 60 mg per day intramuscularly for 10 days, is still thought by many to be the most effective treatment [754]; its possible cardiotoxic action necessitates close observation during its administration.

The second approach is the elimination of amebas from the colon by combined chloroquine-antibiotic therapy. It may be necessary to follow this treatment with a course of carbarsone, or with one of the nonabsorbable arsenic compounds such as Milibis, or with one of the iodine compounds such as Vioform, Diodoquin, or Chiniofon. In resistant cases, repeated courses using more than one drug are necessary. The antibiotic Fumagillin may prove useful for resistant cases.

Finally, amebic abscesses may require surgical drainage, chiefly by needle aspiration [754, 826]. Open drainage should be done only if the abscess is secondarily infected [754]. Any type of drainage should be preceded by a course of chloroquine or emetine [384].

Other Protozoa Which Invade the Liver. The flagellate *Giardia lamblia* commonly occurs in the duodenum, bile ducts, and gallbladder. It probably causes mild clinical symptoms and has been accused of producing a cholecystitis with chole-

lithiasis. It is easily demonstrated in duodenal aspirations [1913].

Trichomonas hominis has been demonstrated in the gallbladder and in liver abscesses but is probably not pathogenic. *Balantidium coli* may invade the liver, as *Endamoeba histolytica* does, and occasional abscesses have been reported [2804].

Coccidium cuniculi produces fibrosis and cirrhosis in rabbits and mice and supposedly in man [2804].

HELMINTH INFESTATIONS OF THE LIVER

Adult worms or ova infest the bile ducts, e.g., ascaris, or the portal vascular system, e.g., schistosoma, or produce hepatic cysts, e.g., echinococcus, cysticercus.

Roundworms (Nematodes)

ASCARIS LUMBRICOIDES. The adult ascaris lives in the small intestine but exceptionally migrates into the bile ducts, where it produces either mechanical obstruction or toxic irritation by excretory products of the living organism or decomposition products of dead ones. This obstruction or irritation may be accompanied by bacterial infection [1620]. Cholangitis and obstruction of the larger ducts and even of the common bile duct by dead parasites, or of the smaller bile ducts with abscesses and subsequent perforation, can develop. Ascaris has also been accused of producing acute cholecystitis, acute dilatation of the gallbladder with lymphadenitis, or gallstones. Rarely, ascaris larvae enter the blood stream and produce visceral granulomas with eosinophilia, for instance, in the liver [2716]. The canine ascaris likewise produces such granulomas.

OTHER NEMATODES. Larvae of other nematodes occasionally produce lesions similar to those of ascariasis [2286]. *Strongyloides stercoralis* may invade the bile ducts and cause obstructive jaundice, cholecystitis, and rarely hepatic granulomas [1892]. *Capillaria hepatica*, a small trematode common in the livers of wild animals, especially rats, occasionally produces small granulomas in the human liver when infested animals are eaten [2161].

Nonsegmented Flatworms (Trematodes)

These parasites are either inhabitants of the bile ducts, with the larvae invading liver tissue, i.e., liver flukes, or inhabitants of the liver itself

in the parenchyma or the portal tracts. They also frequently involve other organs and do not necessarily cause hepatic disorders, i.e., blood flukes.

Liver Flukes. The adult flat liver flukes are between 0.5 and 3.0 cm in length and live in the larger hepatic ducts. The ova are excreted in the stool. The larval stages develop in snails, and infestation occurs via ingestion of fish or vegetables. Various domestic and wild animals are the main hosts, from which spurious infestations of man may occur through ingestion of the livers of these animals. Ascending infestation through the common duct was assumed; but now either invasion of the portal vein through the peritoneal cavity, or invasion of the hepatic parenchyma through Glisson's capsule is assumed, at least for *Clonorchis sinensis* [3061] and *Fasciola gigantica* [3199]. In this migration, the larvae damage the hepatic cells either by trauma or by local toxic action with resulting granulomas. The adult worm obstructs the bile ducts, thus producing diffuse cholangitis, suppuration, and abscess formation. Eventually the periportal tissue, including venous walls, becomes sclerotic, and cirrhosis with ascites and sometimes even carcinoma develops. The cholangitis is characterized by a pseudoepitheliomatous proliferation of the epithelium. The bile ducts are surrounded by eosinophils, lymphocytes, and plasma cells. This inflammatory lesion may heal with scarring. All the flukes described only occasionally invade man and occur rarely in the United States.

CLONORCHIS SINENSIS (CHINESE LIVER FLUKE). The adult worm is 1.0 to 2.5 cm in length and is found mainly in eastern Asia. Fish flesh is the source of infestation. In Japan almost three-fourths of the population are infested, with considerable morbidity and mortality, especially from the complications [1620]. The worm has been found in the Western world only in persons coming from eastern Asia [122, 880]. Excessive proliferation of ductules around larger bile ducts produces adenomalike structures, which obstruct bile flow. This causes cholangitis, hepatic abscesses, and intrahepatic stone formation [1550]. Chronic clonorchiasis is often followed by hepatic carcinoma, mainly of the ductular type [2776].

FASCIOLA HEPATICA (SHEEP-LIVER FLUKE). This worm is 3.0 cm in length and about 1.3 cm in width. The related *Fasciola gigantica* is even larger. Sheep are primary hosts for *F. hepatica*, and cattle and swine for *F. gigantica*. The clinical manifestations are fever, pain, and occasionally

jaundice in the migratory stage [3199]. Invasion of the bile ducts leads to prolonged hepatobiliary disease. Infestation generally occurs following ingestion of contaminated vegetables.

OPISTHORCHIS FELINEUS (CAT-LIVER FLUKE). This trematode is 0.7 to 1.2 cm in length and is found in Europe and Asia. Infestations are observed in fish, and the primary host—the dog, cat, or fox—acquires it by ingesting fish or by drinking contaminated water.

DICROCOELIUM DENDRITICUM (LANCET FLUKE). This worm is 0.5 to 1.5 cm in length and is found in sheep all over the world. Human infestations are uncommon and result mainly from ingestion of infected animal livers.

Blood Flukes (*Schistosomiasis*, *Bilharziasis*)

These flukes produce severe hepatic injury, more severe in young people, brought about by toxic or allergic reactions to the parasites, to their ova, or to their metabolic products. The ova are released in the feces of man, the definitive host. The ova hatch and are short-lived unless they become attached to snails. Free-swimming cercaria develop and penetrate the unbroken skin of man, where they produce an allergic reaction called "swimmer's itch." They follow an intravascular route through the lungs, heart, and mesenteric vessels to the portal vein branches in the liver. During this period of migration, clinical allergic manifestations, with eosinophilia, urticaria, asthma, and hemorrhagic tendencies develop. In the portal veins, the worms grow to a length of 0.5 to 2.5 cm. They reach sexual maturity, copulate, and move back into the branches of the mesenteric veins, where ova are released.

The spread of the disease results from inadequate sewage disposal, for instance from the presence of the snail vector and accumulation of stagnant water in ponds exposed to the sun, for example in irrigation ditches. The migration of Puerto Ricans to the continental United States has made the disease an important problem in this country [2092].

Types of Schistosomal Infestations. *Schistosoma japonicum* migrates into the superior mesenteric veins, and *Schistosoma mansoni* migrates into both the superior and inferior mesenteric veins. The eggs of both worms are extruded into the intestine, thereby infecting the feces. The adult worms do not produce clinical manifestations, although local tissue reactions can occur from death of the worms. Some of the eggs of *S. mansoni* and *japoni-*

cum are carried by the portal vein to its small intrahepatic branches, where they adhere to the vein wall by means of their spine. The endothelium grows around them, and finally they are extruded through the vessel wall into the parenchyma, where they produce a granulomatous reaction. *S. japonicum* occurs in eastern Asia; *S. mansoni* is found in Egypt [1178], South America (Brazil and Venezuela) [1620], and Puerto Rico [1838]. *Schistosoma haematobium*, which occurs in Africa, migrates through the inferior mesenteric veins and through the hemorrhoidal veins to the venous plexus around the bladder. The eggs are extruded into the bladder, and the urine thereby becomes infectious.

Experimental Studies. The lesions of schistosomiasis are readily reproduced experimentally, and much of the information available about earlier stages, especially the stage of migration, is based on these studies. Diffuse hepatic injury with cirrhosis has been produced in monkeys, rabbits, and albino rats. In rats, the bile duct epithelium becomes temporarily hyperplastic [56]. In rats and guinea pigs, the transient cirrhotic changes are associated with obstruction of portal vessels by granulation tissue and with the development of extensive portosystemic collateral anastomoses, which seems to prevent portal hypertension and splenomegaly [1854]. Hepatic function is impaired in animals experimentally infested with *S. mansoni* [728].

Clinical Manifestations. Three stages are conventionally recognized in descriptions from endemic areas such as Egypt [1178], Venezuela [1620], and Puerto Rico [1838]:

1. The early stage of migration and maturation is characterized by skin itching and eosinophilia.
2. The intermediate stage of oviposition in various organs, predominantly the liver, begins on the fortieth day. At this time fever with systemic manifestations is in the foreground, as well as abdominal discomfort with or without pain, urticaria, and cough. Jaundice occurs occasionally, owing to toxic hepatitis or to associated circulatory complications. Hepatosplenomegaly and eosinophilia are present. Subsequently the symptoms become less dramatic and irregular but linger for a long period of time.
3. The third, or late, stage of frank visceral damage is one in which the organ involvement depends on the location of adult worms and especially of ova.

The organs most frequently involved are the colon, the liver and portal veins, and the lungs.

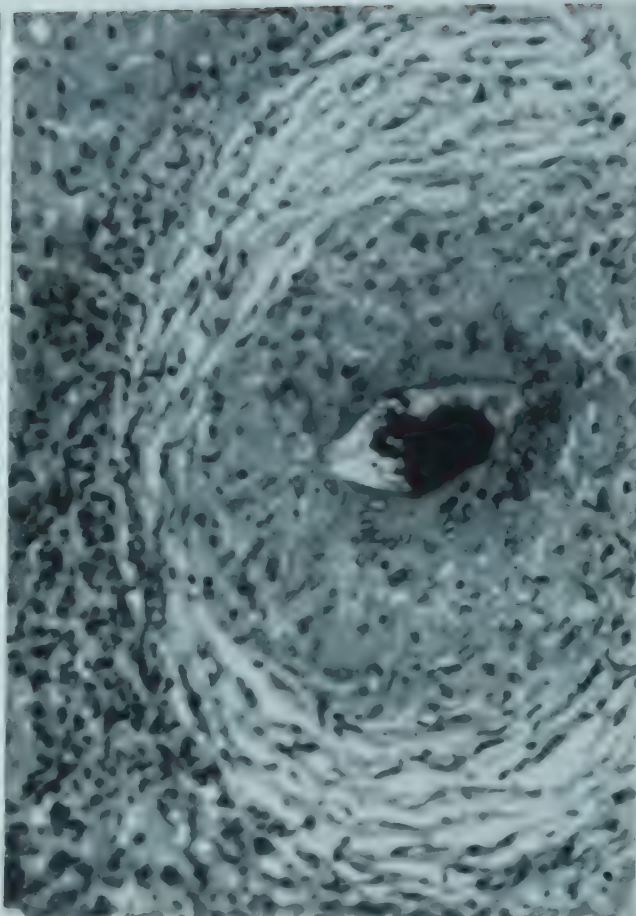
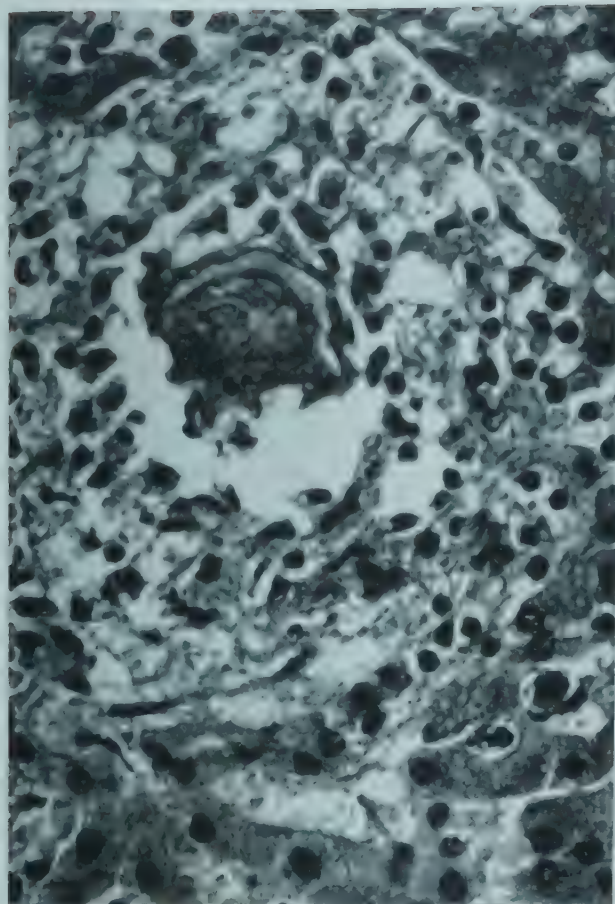


FIG. 185 *Left.* Ovum of *Schistosoma mansoni* with terminal spine in a small branch of the portal vein. The endothelial cells are proliferated, and there is moderate cellular infiltration, chiefly by histiocytes and some eosinophils around the vessel. H&E ($\times 500$). (*Popper, H., and Volini, I. F.: Am.J.Clin.Path.* 19:1146, 1949. Courtesy of The Williams & Wilkins Company, Baltimore.) *Right.* Granuloma composed of epithelioid cells around calcified schistosome in liver biopsy specimen. H&E ($\times 190$).

Proctitis and colitis, sometimes with symptoms of dysentery, characterize the intestinal form of the disease and may complicate the hepatic form, although this usually precedes the hepatic manifestations. Involvement of the lungs produces pulmonary hypertension with cardiac symptoms. Hepatic involvement is prognostically the most important and may become manifest years after the original infection. Hepatic involvement varies from very mild manifestations [2092, 2516] to severe cirrhosis with all its complications [1620, 1838, 3285].

Basically two types of hepatic involvement seem to exist. In the first, the sequelae of portal hypertension are in the foreground as a result of obstruction of portal vein branches in the liver and possibly because of obstruction of mesenteric veins. Splenomegaly and esophageal varices dominate the picture in the absence of other symptoms [2516]. Anemia, if present, is the result of hypersplenism or bleeding varices [2795]. The liver is enlarged and tender. Patients with this condition

may require surgery for the relief of portal hypertension.

In the second type all manifestations of Laennec's cirrhosis may be present, with jaundice, ascites, and hepatic failure. Medical therapy is notoriously unsuccessful; it usually consists of administration of trivalent antimony, in the form of tartar emetic, or Fuadin, both of which are very toxic.

Laboratory Findings. The hepatic tests in early stages do not show consistently abnormal results [2026, 2092]. In the form with portal hypertension, Bromsulphalein retention is especially severe [2795]. In the form with frank cirrhosis, abnormal results may be found in all the hepatic tests. Eosinophilia usually does not persist into the cirrhotic stage.

Structural Alterations. The histologic characteristics of ova and granulomas in the liver have made liver biopsy a valuable method for diagnosis of schistosomiasis, in addition to stool examinations and rectal biopsy [2092, 2516, 3237] (Fig. 185.

right). The ova are characterized by a spine, which is lateral in *S. mansoni*. They are about 0.14 mm in length and are dark if the embryo is dead. Changes in the liver result from one or more of the following factors: (1) local effect of the adult worm, which sometimes can be seen in the earlier stages and is probably insignificant; (2) local effect of ova, which frequently are numerous in or near the finest portal vein branches; (3) systemic, toxic, or allergic effects; (4) contributing factors, especially malnutrition.

NONSPECIFIC REACTIVE HEPATITIS. In the early stages of migration and early oviposition, nonspecific reactive hepatitis is found with hyperemia and focal necrosis.

GRANULOMA FORMATION. Following oviposition in clinically active or inactive cases, reaction to the ova leads to circumscribed focal changes around them. Around usually intact ova in small portal vein branches or in the parenchyma, granulation tissue is found, consisting of histiocytes with a sprinkling of eosinophils and a few neutrophilic segmented leukocytes (Fig. 185, left). Later, lymphocytes appear. Eventually epithelioid cell granulomas containing foreign body giant cells develop around necrotic or calcified ova (Fig. 185, right). They are frequently surrounded by a lymphocytic ring. They are eventually encapsulated by a loose or dense connective tissue and may become fibrotic. These tuberclelike granulomas are usually near the portal fields. The portal tracts become irregularly enlarged and stellate and, in places, connected to one another by fibrotic strands. Thrombophlebitis in larger intrahepatic branches of the portal vein may be found, as well as periphlebitic fibrosis [1992]. Irregularly scattered smaller and larger areas of focal necrosis containing lymphocytes and eosinophils but no ova and not connected with portal tracts are considered to be the result of a systemic reaction of circulating metabolic products [1178, 1620]. Occasionally the Kupffer cells and histiocytes in the portal tracts contain an iron-free pigment histochemically similar to that found in malaria. This pigment is the result of the decomposition of hemoglobin derived from red cells ingested by the parasite [1620, 1838].

PIPESTEM CIRRHOSIS. If the hepatic involvement is severe, obstruction of and fibrosis around the larger branches of the portal vein produce a thick irregular network of white connective tissue. This is also associated with a finer network caused by the scattered perilobular fibrosis which is induced

by the smaller granulomas. The lobular architecture is preserved, but the term "pipestem" cirrhosis has been applied to this condition [3285]. This form, however, seems to be rare [1620] except in the Far East.

SEPTAL CIRRHOSIS. A diffuse septal cirrhosis may be observed in patients with schistosomiasis. The liver may be severely fatty or it may contain no fat; it may also show areas of collapse. This is in contrast to pipestem cirrhosis. The relation of septal cirrhosis to schistosomiasis is unsettled. Since the relatively small number of scattered granulomas hardly explains the diffuse cirrhosis, some investigators feel that septal cirrhosis is caused by nutritional factors common in the tropics [3285]. Others implicate the diffuse toxic effects of the parasite [1178, 1620]. The common occurrence of hepatic carcinoma in the Caribbean area has been associated with the presence of schistosomiasis [240].

Segmented Flatworms (Cestodes)

Adult tapeworms, *Taenia saginata*, rarely migrate into the bile ducts. They have been found in exceptional instances in the gallbladder, where they supposedly produced cholecystitis and stones [1996].

Taenia solium, the adult of which also inhabits the small intestine of man, usually infests the hog, where encysted larvae form large cysts in various organs, including the liver. Occasionally this cyst stage, cysticercus, is also noted in the human liver, but usually it is only an accidental lesion.

Echinococcus Cyst (Hydatid Disease)

The adult tapeworm, *Taenia echinococcus* or *granulosa*, is only 3 to 6 mm in length and inhabits the intestines of dogs and other canines. The eggs excreted with the feces are ingested by sheep, cattle, and, exceptionally, pigs, which serve as hosts for the larval stage. The larvae penetrate from the intestinal tract into the viscera and form cysts in various organs, but predominantly in the liver. In these cysts new embryos are formed asexually.

Dogs are infested by ingestion of infested meat, mainly from sheep. In countries in which dog as well as sheep infestation is frequent, man may also serve as an intermediate host. In the United States dog infestation is extremely rare, and therefore infestation of man is exceptional. It is seen almost exclusively in immigrants from countries in which these diseases are prevalent, such as Germany, the

Balkans, Iceland, Australia, New Zealand, Holland, and South America [943, 2804]. Sanitary measures have reduced its incidence in various countries, such as Iceland and Australia, and therefore extensive descriptions are found mainly in the earlier European literature [943, 2804]. Recent American publications report isolated cases [88, 2623, 3658].

Clinical Manifestations. Clinical manifestations, if any, usually develop many years after the original infestation. They occur most often in childhood, or in women because of closer contact with pets, especially dogs. The cysts grow very slowly, and pressure symptoms do not develop. In one patient, a liver weighing 10,000 gm produced no clinical manifestations except for the huge size, which was noticed by the patient. Symptoms occur if a complication of the disease develops. The most common complication, rupture of the cyst, occurs in 35 per cent of cases. The complications are:

1. Echinococcus cyst fluid may leak into the blood stream, causing allergic reactions and even anaphylactic shock [119, 943].

2. Encroachment upon the bile ducts, either by pressure from outside or by rupture of a daughter cyst into a bile duct, results in jaundice [1195, 2623]. Secondary bile duct infection and symptoms simulating choledocholithiasis occur, also associated with allergic manifestations and eosinophilia [119, 2623].

3. Cysts become secondarily infected. Necrosis and nonspecific reactive hepatitis develop, sometimes causing jaundice. Infection is the main cause of pain, fever, and leukocytosis in the absence of jaundice.

4. Bizarre manifestations result from rupture of cysts into various organs, including the peritoneum or even the adrenal gland [943, 1195, 2804]. Hematogenous dissemination has also been suggested.

5. Many small exogenous cysts can develop which burrow into the surrounding tissue (alveolar echinococcus). This type of infestation acts almost like a tumor, producing obstructive jaundice (see Alveolar Echinococcus, later in this chapter).

6. Compression of vessels at the hilus of the liver leads to atrophy of the left lobe [1572].

7. Carcinoma of the liver has been reported, giving rise to the opinion that the parasites or their products are carcinogenic.

Laboratory Findings. The results of the flocculation tests are normal, no jaundice is present, and

serum-alkaline phosphatase activity is slightly increased in the uncomplicated cases. Laboratory tests pertaining to echinococcus disease are (1) the precipitin test, which is positive in 65 per cent of cases; (2) the complement-fixation test in serum, which is positive in 60 per cent; (3) the intradermal skin test, which is positive in 90 per cent [1996]; (4) high eosinophil count, which occurs in about 25 per cent [943, 2623]; (5) roentgenologic demonstration of cysts with calcified walls [943, 1444, 2563]. Echinococcus cysts are a common cause of radiologically apparent calcifications in the liver, although very large cysts without any calcifications have been seen.

Structural Alterations. STAGE OF MIGRATION. The larva which hatches from the egg in the intestine migrates into the viscera via the blood stream. The liver is the site of infestation in at least 70 per cent of cases [88, 2804]. In about 80 per cent of these cases, the right lobe is involved, reflecting the streamlines in the portal system from the upper intestinal tract. The larva is transformed into a cyst with an outer, chitinlike, lamellated layer, or ectocyst, and an inner germinal layer, or endocyst. The surrounding tissue of the host forms a capsule of collagenous connective tissue, the pericyst. Unilocular cysts 1 to 20 cm in diameter and usually with a smooth wall may develop, distorting and enlarging the liver tremendously (Fig. 186, upper left).

FERTILE STAGE OF ENCYSTMENT. During the fertile stage, cells develop in the germinal layer, giving it a granular appearance. These cells give rise to embryonal heads of the tapeworm, scolices, up to 300 μ in size. Small organs develop, the most characteristic of which are hooklets (Fig. 186, lower left). Hydatid fluid fills the cyst lumen. It is almost protein-free but contains highly irritating material responsible for the violent reaction upon its entrance into the circulation following rupture, leakage, or incision. In the unilocular cyst, the lining membrane shows multiple invaginations into the lumen. These are also lined by the same cell-producing germinal layer. This layer also forms brood capsules, which gradually enlarge to form endogenous daughter cysts, since they lie in the lumen of the now much larger parent cyst (Fig. 186, lower right). These processes of continuous asexual formation of scolices, invagination of the original cyst with resulting brood capsules, and formation of thousands of daughter and grand-daughter cysts may go on for many years. The cyst

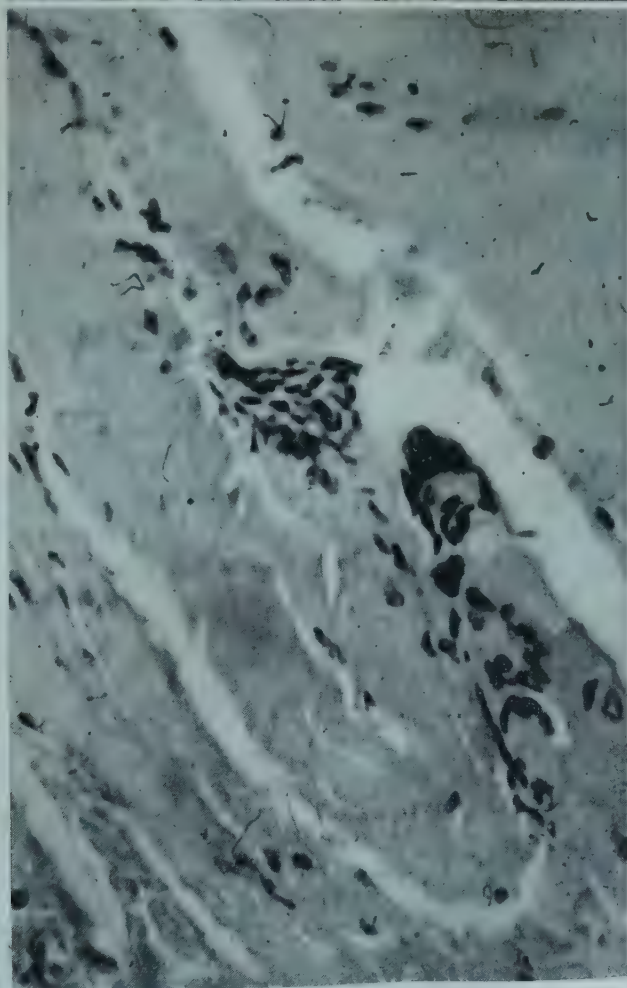
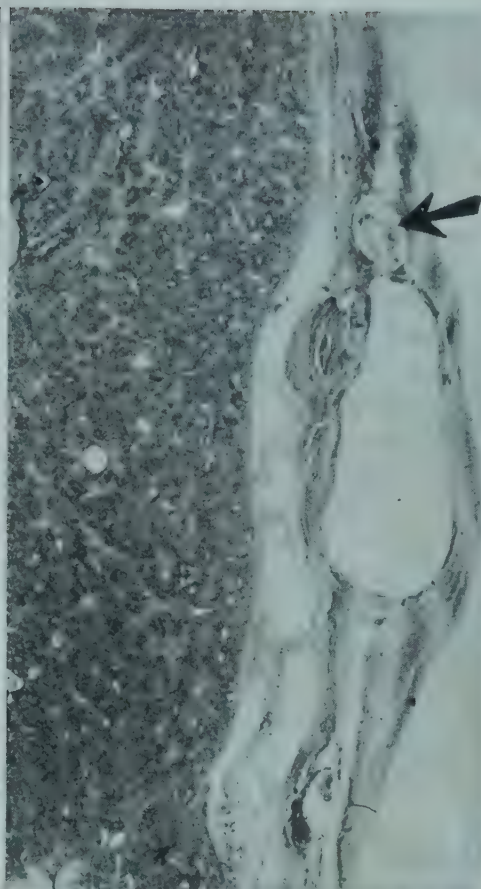


FIG. 186 *Upper left.* Echinococcus (hydatid) cyst enlarging left lobe of liver. *Upper right.* Partially sterile cyst with daughter cysts (arrow). Mallory's aniline blue ($\times 3$). *Lower left.* Scolices in fertile portion of cyst. H&E ($\times 230$). *Lower right.* Free cysts in large hepatic cyst.

appears to contain granules, or hydatid sand, in which hooks can be clearly discerned. This slow and usually asymptomatic process leads to cysts as large as a child's head, which may still produce scolices and cystic invaginations although they are many decades old.

STERILIZATION OF CYSTS. The asexual formation of scolices may stop, and the entire cyst or part of its circumference may become sterilized. This process is associated with invasion of the parasitic layers of the cyst by the connective tissue of the host, so that eventually a smooth cyst wall composed of fibrous tissue results (Fig. 186, upper right). Smaller cysts can be completely obliterated. This process is usually associated with calcium deposition.

INFLAMMATION IN AND AROUND CYSTS. Inflammatory reactions around the cyst, with infiltration of lymphocytes and histiocytes, probably result from leakage of hydatid fluid. Granulation tissue forms, and the cyst often becomes obliterated. Cysts are sometimes secondarily infected by the hematogenous route.

FORMATION OF EXOGENOUS DAUGHTER CYSTS. Occasionally external outpouchings form exogenous daughter cysts, either in the wall of the parent cyst or in the surrounding hepatic tissue. The resulting multilocular cysts vary in size, and up to 12 large cysts may be seen in the liver. Exogenous daughter cysts can also be implanted into the peritoneal cavity, into omentum and mesentery, with further spread.

Alveolar Echinococcus. Excessive formation of small daughter cysts on the surface of the original cyst, usually very small in size, is characteristic of alveolar echinococcus. It occurs in countries other than those in which unilocular cysts are found, such as Southern Germany, Tyrol, and Russia [943, 1996, 2804]. This has given rise to the opinion that this type of proliferation of the cysts reflects a different parasite. Functionally and structurally, the extreme proliferation of cysts filled with gelatinous material acts like a tumor, extending into the lymphatic vessels and even producing metastases. The alveolar echinococcus was once considered a peculiar type of mucus-producing carcinoma. Clinically, cachexia and jaundice are common. In contrast to the unilocular or multilocular echinococcus, the alveolar type has a grave prognosis and is rarely controlled surgically [3540].

ARTHROPODS IN THE LIVER

In the liver and also in the spleen, hard yellow nodules up to 3 mm in diameter can be found which are easily shelled out and which are often incorrectly considered old tuberculous lesions. They represent the reaction to the larvae, *Pentastomum denticulatum*, of an arachnid, *Linguatula rhinaria*, living in the noses of various animals, especially the dog. The eggs reach the human intestinal tract from grass and migrate to the liver, where they firmly encapsulate and calcify. They have no functional or clinical significance.

VASCULAR DISEASES OF THE LIVER

Diseases of the blood vessels produce many functional and structural changes in the liver. Most manifestations of vascular disorders have been discussed under other headings; in this chapter only reference is made to them. Also, the hepatic and extrahepatic sequelae of ligation of the main hepatic blood vessels have been discussed previously in detail (see Chap. 18).

Diseases of the Portal Vein

Congenital anomalies of the portal vein have been discussed under structure of the portal vein (see Accessory Portal Veins and Anomalies of the Portal Vein, under The Portal Vein, Chap. 17). Acute and chronic portal vein thrombosis, including cavernomatous formation of the portal vein and Cruveilhier-Baumgarten disease, have been discussed (see Portal Vein Thrombosis, also Cruveilhier-Baumgarten Disease, under Infrahepatic Portal Hypertension, Chap. 29). Phlebosclerosis [1989, 2359], which may proceed to extensive atheroma formation with calcification [3590] and which is frequently found in portal hypertension, may precede it and may actually be a cause rather than a result of hypertension. Primary portal sclerosis caused by primary inflammatory, degenerative, or nutritional factors has been described [963, 1989, 2733]. Previously, tuberculosis and syphilis were considered important causes of vascular changes [2733].

Suppurative Pylephlebitis. Suppurative pylephlebitis is usually associated with thrombosis of the portal vein or its tributaries and is related to purulent hepatitis (see Purulent Biliary Hepatitis, under Infected Biliary Hepatitis, Chap. 47) and hepatic abscesses (see Liver Abscesses, later in this chapter). The lesion involves either the main

stem of the portal vein or its branches in the liver. Sometimes both the main stem and the branches are involved because of dissemination of infected emboli from the portal vein thrombus. The etiology and the clinical manifestations of intrahepatic and extrahepatic suppurative pylephlebitis are similar.

ETIOLOGY. The portal vein becomes infected from (1) appendicitis and suppurative thrombophlebitis of the appendiceal veins, formerly the most important cause; (2) extrahepatic cholangitis, either directly or via suppurative lymphadenitis of the portal lymph nodes, phlegmonous infiltration of the perivenous connective tissue, or perforation of a stone; (3) cholecystitis, either by direct spread into the liver or via cholangitis or thrombophlebitis; (4) hepatic abscesses or purulent hydatid cysts; (5) carcinomas of the pancreas; (6) inflammatory gastrointestinal diseases, such as regional ileitis [3304], ulcerative colitis [3358], diverticulitis, or infected and thrombosed hemorrhoids; (7) splenic vein thrombosis; (8) exceptionally, infections of the male or female genital tract [281, 2502]. Most commonly *Escherichia coli* and streptococci are the offenders. The recent progress in antibiotic therapy has greatly reduced the incidence of this condition; it is now mainly encountered as a terminal process found incidentally at autopsy.

CLINICAL AND LABORATORY MANIFESTATIONS. Sepsis is observed, with fever, chills, epigastric pain, increasing abdominal distention, a large and tender liver, and, usually, an enlarged spleen. Vomiting often occurs. Formerly, when appendicitis was the etiologic factor, jaundice was not in the foreground, having been noted only in 30 per cent of the cases. At present, when biliary tract infections are a more common cause, jaundice is almost regularly found. Ascites is usually present and

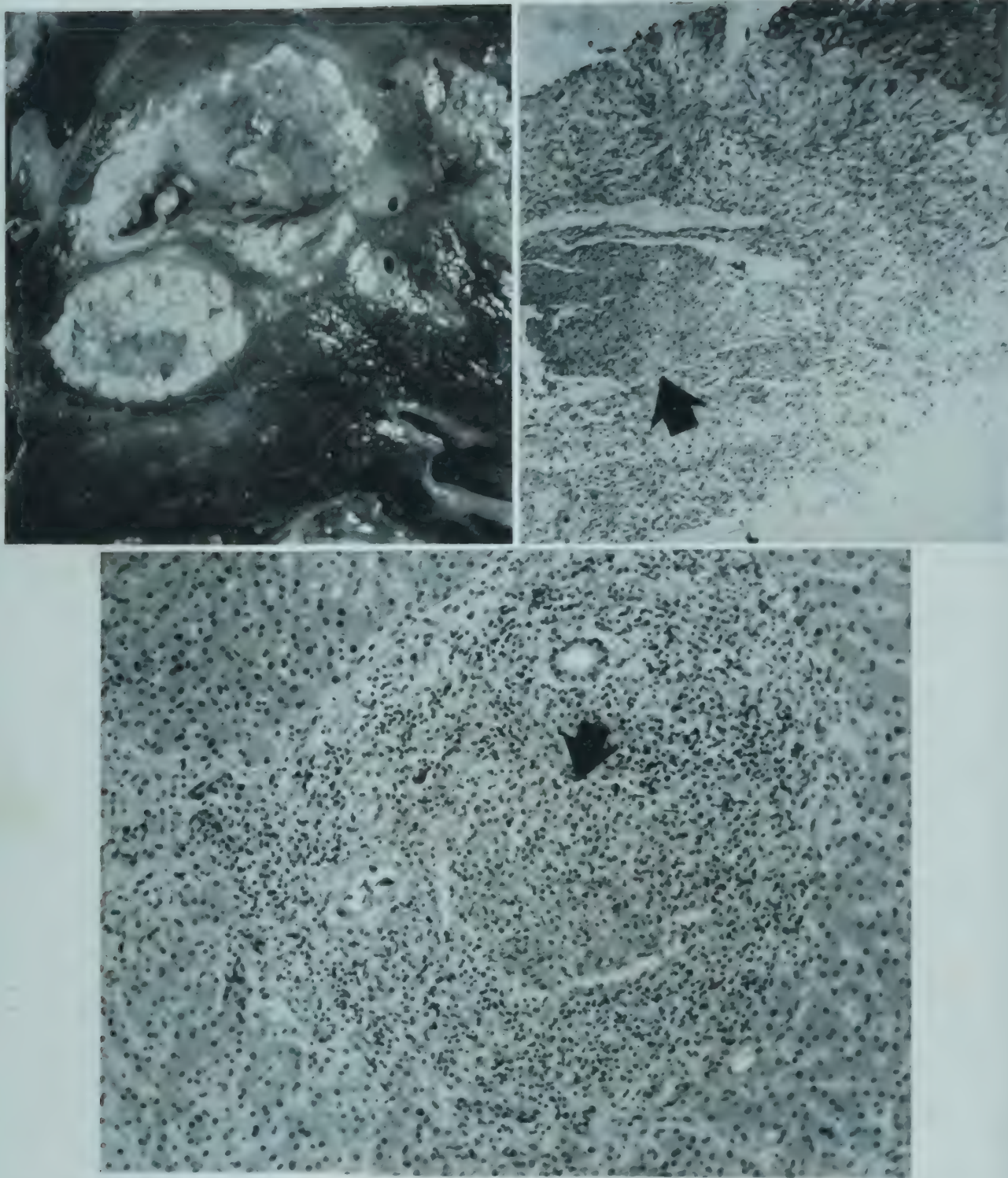


FIG. 187 Upper left. Pylephlebotic liver abscess. Upper right. Purulent pylephlebitis with thrombosis (arrow) in biopsy specimen. H&E ($\times 60$). Lower. Autopsy specimen showing purulent thrombus (arrow) in small portal vein branch, with beginning suppuration in the portal tract. H&E ($\times 110$).

depends on the degree of portal vein obstruction. Gastrointestinal bleeding from esophageal varices is common. If the condition is not a preterminal complication, its duration varies between weeks and months before the fatal outcome. Recovery is now reported more frequently [2585]. The results of the tests reflecting hepatocellular damage are usually abnormal, and those showing cholestasis depend on the underlying disease. In most in-

stances, the manifestations of purulent hepatitis dominate the clinical picture. Portal vein obstruction has been demonstrated by percutaneous portosplenography, and surgical ligation of the involved portal vein has been recommended [3643].

STRUCTURAL CHANGES. If the main stem is involved, its wall is thickened and the inner lining is succulent, dark red, and covered by adherent purulent thrombi. Histologically, the intima, media,

and adventitia are infiltrated by inflammatory cells, with a varying admixture of segmented leukocytes. When smaller and larger intrahepatic tributaries are involved, fibrin thrombi partially or completely obstruct their lumens, and they are infiltrated by pus cells (Fig. 187, upper right). The wall shows similar infiltrations, which extend into the portal tracts (Fig. 187, lower). In the surrounding parenchyma, the sinusoids may be distended by a fibrin network around segmented leukocytes. The hepatic cells are frequently necrotic. Portal vein thrombosis may lead to perihepatic and subphrenic abscesses. Pulmonary and brain abscesses are rare. Thrombophlebitis of the hepatic veins has been said to be necessary for the escape of bacteria into the systemic circulation in suppurative pyelphlebitis. For this reason, blood cultures are rarely positive. Umbilical infections in newborn babies, a special variety of pyelphlebitis, lead to hepatic abscesses [2347] (see Umbilical Sepsis in the Neonatal Period, under Nonspecific Reactive Hepatitis, Chap. 41).

Diseases of the Hepatic Artery

Obstruction of the hepatic artery or its branches by experimental or surgical ligation, or by emboli or thrombi, has been discussed (see Hepatic Artery, Chap. 18). Diseases of the hepatic artery usually reflect a generalized arterial disease.

Arteriosclerosis. Systemic arteriosclerosis involves the extrahepatic branches with the same frequency as the mesenteric arteries but far less frequently than the splenic artery. The intrahepatic branches show arteriosclerosis mainly in hypertension. Thickening of the media of the small arteries in the portal tracts is often seen in liver biopsy specimens in patients with hypertension and only exceptionally in nonhypertensive diffuse arteriolosclerosis.

Arteriolo sclerosis. In malignant hypertension, visceral necrotizing arteritis involves the hepatic arteries less frequently than other organs [1996], especially the pancreas.

Hypersensitivity Angiitis. In nonspecific angiitis caused by hypersensitivity [2751, 3698], small hepatic artery branches occasionally exhibit segmental necrosis with thrombosis. Portal vein branches participate less frequently, and hepatic vein branches almost never do.

Polyarteritis Nodosa. In polyarteritis nodosa, the hepatic arteries participate in approximately two-thirds of the cases [94, 2369]. Involvement of the gallbladder vessels is almost as common. The dis-

ease involves not only smaller arterioles but also larger, grossly visible arterial branches and occasionally portal vein branches. The lesion is characterized by an initial segmental fibrinoid degeneration, often with leukocytic infiltration, which is soon followed by reactive proliferation of the intima and periarterial granuloma-like infiltration. Thrombotic occlusion of the lumen leads to hepatic infarcts, visible in approximately 15 per cent of cases [2369]; often the occlusion is not demonstrable (Fig. 188, top). Aneurysms of the hepatic artery, which often result from polyarteritis, are discussed in the following section. A nonspecific inflammatory reaction in the portal tracts also occurs in polyarteritis [2369], apparently as a reflection of the toxicity characteristic of the disease. Cirrhosis occasionally observed in this condition is probably not related to it.

Clinical and functional manifestations are infrequent. Hepatomegaly is found in about 20 per cent and icterus in about 10 per cent of cases [1389, 2369], but mostly in terminal stages owing to cardiac failure. Exceptional cases have been seen in which polyarteritis nodosa presented itself primarily as a hepatic disorder [2369].

Aneurysms of Hepatic Arteries. Aneurysms of the hepatic artery are rare and occur more frequently in the extrahepatic than in intrahepatic branches. They are usually multiple and vary in size from pinpoint up to cherry-size [162, 870, 1250, 2676].

ETIOLOGY. Some hepatic artery aneurysms result from polyarteritis. Supposedly 50 per cent are caused by infections such as cholangitis or cholecystitis [2676]. A small number are produced by trauma. Arteriosclerosis may play a role, but syphilis apparently does not.

CLINICAL MANIFESTATIONS. Some aneurysms never lead to symptoms and are found incidentally at autopsy or surgery. Some perforate into the free abdominal cavity [2804] or, rarely, into bowel, gallbladder, or portal vein. Most often they perforate into biliary ducts, resulting in gastrointestinal hemorrhage, the origin of which is difficult to diagnose (Fig. 188, center and bottom). Typical symptoms are right upper abdominal colicky pain, jaundice, and melena [3432]. Jaundice results from biliary obstruction by pressure exerted by the aneurysms or by blood clots after rupture.

TREATMENT. The ideal treatment is excision or wiring of the aneurysm [1250]. If this is not possible, the hepatic artery must be ligated, especially in cases of bleeding from the aneurysm.

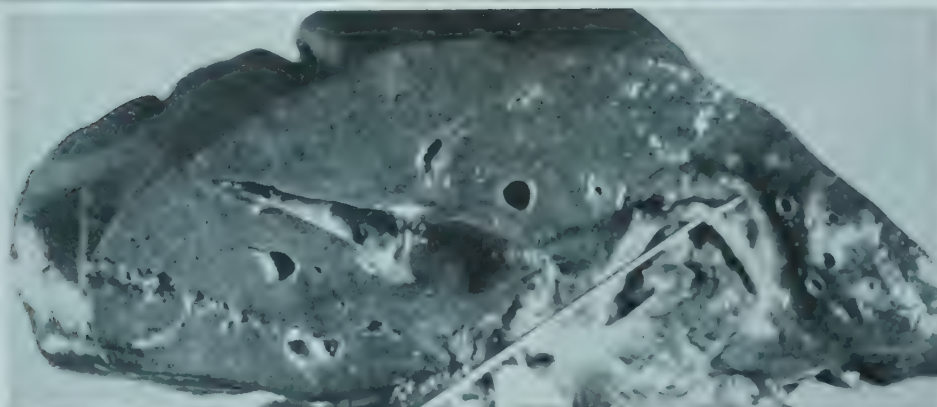


FIG. 188 Top. Anemic infarct of left lobe of liver in polyarteritis nodosa. Center. Large diverticulum of the hepatic artery communicating with the left hepatic duct. Bottom. Communication of the incised diverticulum with the left branch of the hepatic artery (large arrow). Opening of the cystic duct into the slightly dilated common duct (smaller arrow). B&C (Vulliamy, F., and Popper, H. *Scd North America* 28:262, 1948).

Other Arterial Diseases. In disseminated platelet thrombosis the hepatic vessels are frequently but apparently only terminally involved [1231]. In rare instances of lupus erythematosus, the hepatic arteries are the site of a severe arteritis. Exceptionally the hepatic arteries are diseased in generalized obliterating thromboangiitis [1417].

Diseases of the Hepatic Veins

The hepatic veins show phlebosclerosis in the presence of increased venous pressure, as in constrictive pericarditis. Thrombosis is clinically more important and occurs in polycythemia vera [3309] or visceral thrombophlebitis migrans [1149], in both instances as part of a generalized systemic disease. Exceptionally, retrograde embolism occurs. Tumors, gummas, and cysts sometimes compress the hepatic veins, and strictures develop in constrictive pericarditis (see Hepatic Vein, Chap. 18). The most important obstruction is obliterative endophlebitis, or Chiari's syndrome (see Occlusion of the Hepatic Veins—Chiari's Disease, Chap. 48).

Alterations of the Sinusoids

The appearance of the liver in autopsy specimens often reflects alterations of the blood content which are mostly terminal and brought on by spasms or dilatations of afferent vessels. The irregular mottling caused by these variations in blood content is seen in cadaver livers and is also seen during life. Hyperemia of the lobular periphery results from variations in the tonus of the vessels [35]. Occasionally, circumscribed areas are seen which are lighter in color than the surrounding parenchyma, especially in septicemia. On the cut surface, the boundaries of these areas are sometimes straight and sometimes irregular. These light "anemic" spots, also called "septic liver spots," probably result from circumscribed vascular spasms, which cause not only local anemia but also circumscribed fat deposition [1366].

PELIOSIS. In patients with advanced pulmonary tuberculosis and occasionally in other conditions, grossly visible circumscribed red foci, varying in size up to 1 cm in diameter, are observed [3525, 3688]. These purplish-red spots, which shine through the capsule, consist of blood cysts and have been called "peliosis." They are not always lined by sinusoidal endothelium, and they communicate with the surrounding sinusoids and sometimes even with central and sublobular veins. Smaller cysts seem to merge with larger ones, and the surrounding hepatic parenchyma shows com-

pression as well as degenerative changes. Alterations in the uninvolved hepatic parenchyma are in keeping with the underlying disease, especially in advanced tuberculosis. Focal necrosis and fibrosis are common. Several theories explain peliosis as the result of vascular changes and necrosis caused by the underlying disease [3525, 3688]. However, the occurrence of similar sinusoidal dilatations in experimental animals with endocrine tumors [1112] suggests a hormonal etiology comparable to the vascular changes occurring in hyperestrogenism and pregnancy.

LIVER ABSCESES

Liver abscesses are produced by pyogenic microorganisms which have entered the liver by various routes. Cytolysis without predominance of pus cells in amebiasis, carcinoma, tuberculoma, actinomycosis, and gumma and alveolar echinococcus cysts resembles an abscess grossly as well as clinically because of the similar palpatory findings and often similar systemic reactions, such as fever, pain, and leukocytosis, and because the hepatic tests indicate a space-occupying lesion. However, these are not really abscesses in the morphologic sense of the word.

Etiology. The most frequently found bacteria in hepatic abscesses are *Escherichia coli*, streptococci, and staphylococci, or combinations of the three [1038]. Many other microorganisms have been isolated, such as Friedländer's bacillus [328], typhoid bacilli, and anaerobic organisms. In almost half the cases, the abscess is sterile [1038]. The routes of infection are the portal vein, the bile ducts, the hepatic artery, direct extension from neighboring structures, direct infection following trauma, and the hepatic vein. In some instances the source can not be established even by necropsy; in these instances, arterial spread from small inconspicuous foci must be assumed [1038].

Types of Abscesses. Abscesses are multiple, multilocular, or solitary. Some of the solitary abscesses result from coalescence of smaller abscesses and then assume a leaflike shape on the cut surface. Small multiple lesions found at autopsy develop terminally in fatal septic or pyemic infections and therefore represent an incidental finding of no clinical significance. Multiple abscesses carry a graver prognosis than single ones, although the prognosis is generally poor, and mortality rates range from 50 to 90 per cent [1038]. Recent

progress in antibiotic therapy may improve the prognosis.

Pylephlebitic Abscesses

Pylephlebitic abscesses are the most frequently found type. They are a more advanced stage of purulent hepatitis and of suppurative pylephlebitis. The portal venous route of invasion is proved by the presence of a suppurative lesion in the portal system. Suppurative thrombophlebitis can frequently be demonstrated, either near the site of the original infection in a mesenteric vein, for instance, or in the main stem of the portal vein or its intrahepatic ramifications. Wide areas of the portal vein system are often intact between thrombotic portions, suggesting embolic transmission from larger vessels into a tributary. The abscess varies in size (Fig. 187, upper left). The pyogenic membrane in earlier lesions is gray-red. In older lesions it is bright yellow, owing to the presence of abundant fat-laden foam cells in the surrounding granulation tissue. In still older lesions, the granulation tissue has become fibrous, and septums extend into surrounding parenchyma. Fibrosis and inflammation are found in portal tracts, with pressure atrophy around the abscess. Portal vein branches in the vicinity contain thrombi with or without suppuration. Bile ducts can become eroded, obscuring the differentiation from cholangitic abscesses. The sources of infection are the same as in suppurative pylephlebitis (see Suppurative Pylephlebitis, earlier in this chapter).

Cholangitic Abscesses

Cholangitic abscesses occur most frequently in association with biliary obstruction and jaundice or at least with infection in the biliary tree. They are preceded by infected biliary hepatitis (see Infected Biliary Hepatitis, Chap. 47). The infection usually spreads via a hematolymphatic route, rather than through the bile ducts, which are commonly only secondarily involved. Spread throughout the liver from an abscess already established occurs through the bile ducts. The bile duct involvement is reflected in the grossly and microscopically recognizable gold-green color of the pus or pyogenic membrane (Fig. 104, bottom).

Cholangitic abscesses frequently complicate internal biliary fistulas and prolonged incomplete biliary obstruction from choledocholithiasis or strictures. They are terminal complications in carcinoma of the pancreas or of the common duct. *Ascaris lumbricoides* invasion of the bile ducts

may produce cholangitic abscesses in children [2797] (see *Ascaris lumbricoides*, under Helminth Infestations of the Liver, Chap. 55).

Other Abscesses

ARTERIAL EMBOLIC ABSCESES. These abscesses, usually small and irregularly spaced throughout the parenchyma, are chiefly the result of arterial emboli originating from a suppurative focus anywhere in the body, such as tonsillitis, osteomyelitis, or puerperal infection. They are usually part of a systemic pyemia, and similar abscesses are found in lung, kidney, and myocardium and may be associated with endocarditis. With increasing control of pyemia by antibiotics, this lesion is becoming rare.

ABSCESS BY DIRECT EXTENSION. Suppurative processes in the gallbladder or in the subphrenic space and other intraperitoneal abscesses may extend into the liver. Perforated peptic ulcers may cause digestion and subsequent suppuration of the adjacent liver parenchyma.

POSTTRAUMATIC ABSCESES. Penetrating traumatic injuries such as stab or bullet wounds and lacerations often produce hepatic abscesses. Abscesses also develop after blunt, nonpenetrating injuries, i.e., "primary hepatic abscess."

RETROGRADE EMBOLIC ABSCESES. Exceptionally, retrograde embolic infection of the liver parenchyma follows suppurative thrombophlebitis of the hepatic vein. This has been explained by a sudden increase of the hepatic vein pressure, such as occurs during coughing [564].

Clinical Manifestations of Hepatic Abscesses. The main clinical manifestations of hepatic abscess are pain in the right upper quadrant, chills, and fever usually described as being of the "picket-fence" type. The liver is enlarged and tender. Splenomegaly is not constant, and jaundice is not always present. Leukocytosis is found, especially in the acute cases, and is a point of differentiation from amebic abscess. Roentgenologic examination demonstrates elevation and fixation of the diaphragm. Cholangiography has also been employed [1909].

The most frequent complication is perforation (1) into the abdominal cavity, with secondary peritonitis; (2) into the pleura; (3) into the pericardium [3725]. Treatment is surgical where possible, through either an anterior or a retroperitoneal approach [1038], supported by therapy with broad-spectrum antibiotics if the offender is not known.

PART VI

*Tumors of the Liver
and Biliary Tree*

HEPATIC HAMARTOMAS

Hamartomas are tumorlike malformations which occur frequently in the liver in various forms. The simple and homogeneous morphologic structure of the liver results from a mutually organizing influence of mesenchyma, in the form of the capillary, or sinusoidal, plexus and collagenous connective tissue, on one hand and entodermal and possibly also mesodermal epithelium on the other (see Structural Organizers, under Embryology, Chap. 20). Disturbances of the effect of these organizers result in various abnormalities of structure [2335]. These improperly organized structures, hamartomas, straddle a line between malformation and tumor, in that the malformed tissue sometimes exhibits tumorous qualities. The tumor growth is limited, and malignant transformation of the hepatic hamartomas rarely, if ever, occurs. Malignant transformation of cysts of the liver has been specifically denied. On the other hand, hamartomas are not usually encapsulated and are sometimes poorly demarcated. Many are only anatomical curiosities detected during operations, autopsies, or, by chance, in biopsies. They are rarely large enough to be felt or to produce the effect of a space-occupying lesion or of obstructive jaundice by pressure on the bile ducts. When they are large enough to have such effects, they become surgical problems. These hamartomas consist either of bile ducts, of mixed elements, or of blood vessels (so-called hemangiomas). To differentiate them from other and usually malignant epithelial tumors of the liver, all small nodules found in the liver at the time of surgery deserve evaluation by frozen section [2321].

Bile Duct Hamartomas

Disturbed differentiation of bile ducts is frequent, resulting in an irregular arrangement of the

ducts sometimes in the form of small nodules or cysts.

Multiple Microhamartomas. Small and irregular proliferations, mainly of perilobular ductules, and also small septal bile ducts in some portal tracts, or even independently of them, are occasionally observed. The ductules and ducts are surrounded by a dense mass of collagen fibers merging with their basement membrane (Fig. 189, upper). They form a plexus of irregularly arranged connecting cavities which communicate with the bile duct system, draining the parenchyma. They may contain bile or small calculi [2153]. These structures form by segmentation and fusion of excess ductal and ductular structures formed during embryonal development [2263, 2456], and they persist, owing to disturbed organizing influences. In an individual lesion, especially if seen in a biopsy specimen, the differentiation from inflammatory bile ductal or ductular proliferation is not easy, and moreover, these lesions have been found associated with diffuse portal inflammation. The proliferated ductules can be differentiated from hepatic cells and interlobular ducts by histochemical reactions. Differentiation of ductules from hepatic cells and connection with ducts can be noted [2263]. The lesions are functionally insignificant and usually are only microscopic features. They may become large enough to be recognized with the naked eye as gray-white ramified strands enforcing the portal tracts and exaggerating the lobular markings [2153, 2263]. Terms such as "multiple bile duct adenomas" or "Meyenberg complexes" [1582] have been used, but the term "multiple microhamartomas" better emphasizes the hamartomatous nature. Clinical symptoms do not occur. The lesion is related to the polycystic liver.

Hepatic Cysts. Sometimes hamartomatous cavities become large and cystic. The cysts often com-

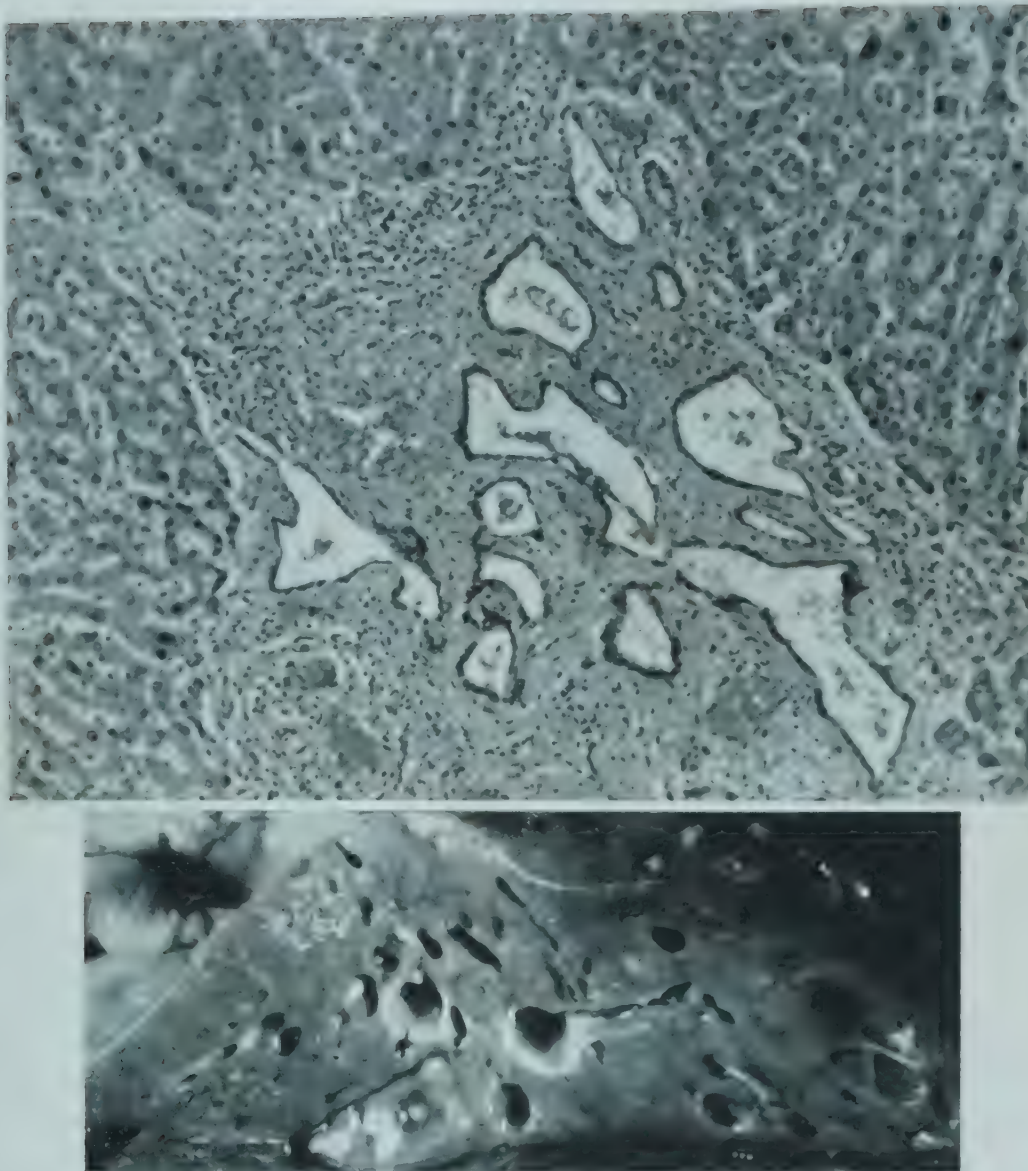


FIG. 189 *Upper.* Microhamartoma (Meyenberg complex). Irregularly proliferated bile duct structures in enlarged and fibrosed portal tract. H&E ($\times 95$). *Lower.* Polycystic disease of the liver.

municate with one another [2456]. They vary in size and number, the larger ones being found mostly in adults. Occasionally single cysts become very large. Scattered cysts sometimes contain papillary structures that have been wrongly called bile duct adenomas or cystadenomas [1470, 1831]. Polycystic disease of the liver (Fig. 189, lower) is usually associated with polycystic disease of the kidney, at least in 50 per cent of the cases, and of the pancreas, and sometimes also with other anomalies, such as aneurysms of cerebral arteries [2263]. The cysts, like the microhamartomas, develop from noninvolution of excess intrahepatic ductules, their development being analogous to the histogenesis of the polycystic kidney. In three-dimensional reconstructions, the communicating intrahepatic bile ducts appear distorted, dilated, and only occasionally segmented and separated from the biliary tree

[2335, 2456]. Communication with the biliary tract is also indicated by the presence of bile casts in the smaller cavities [2263]. The lesion is often familial and is usually diffuse.

CLINICAL MANIFESTATIONS. Symptoms referable to polycystic liver appear, if at all, in the fourth and fifth decades. They consist of upper abdominal pain, rarely severe, with a feeling of fullness. The cysts occasionally can be felt in the enlarged liver. Functional impairment does not occur. The health of the patient is mainly influenced by associated polycystic disease of the kidney [634]. Liver biopsy by needle may be misleading, but the condition has been diagnosed by peritoneoscopy [2443]. Treatment is not required, and malignant degeneration seems to be very rare [3616]. Solitary cysts of the liver, mainly in the inferior portion of the right lobe, may become so large as to pre-

sent a surgical problem [494, 2380]; in such cases, biliary obstruction with jaundice is sometimes present [2380].

STRUCTURAL ALTERATIONS. The cysts are lined by mature bile duct cuboidal epithelium, which is often desquamated. Their walls are composed of cellular connective tissue containing many irregularly arranged bile ducts, often with accompanying vessels and even rudimentary liver lobules. Cysts become up to 20 cm in diameter. The lumen of the cysts contains a clear, watery, yellow fluid with little, if any, bile.

DIFFERENTIAL DIAGNOSIS. Bile ducts in the cyst wall characterize these cysts as bile duct hamartomas. However, the origin of some hepatic cysts can not be established. In the differential diagnosis of cysts, several possibilities have to be considered [1470, 2380]. They are listed in the order of incidence:

1. Parasitic cysts
2. Hamartomatous cysts
3. Degenerated blood cysts
4. Cysts caused by bile stasis
5. Ciliated cysts derived from misplaced intestinal entoderm
6. Teratoid or dermoid cysts
7. Lymphatic cysts

Solid, or Mixed, Hamartomas

Nodules, usually solitary, differing in texture and color from the surrounding liver tissue are frequently found in otherwise normal livers. They vary in size from small, barely visible nodules to large masses several inches in diameter. Tumors weighing 1,575 gm have been reported [2540]. They have no specific predilection and therefore occur more frequently in the larger right lobe. They are usually well demarcated grossly and are sometimes prominent over the surface of the liver; they may even be pedunculated.

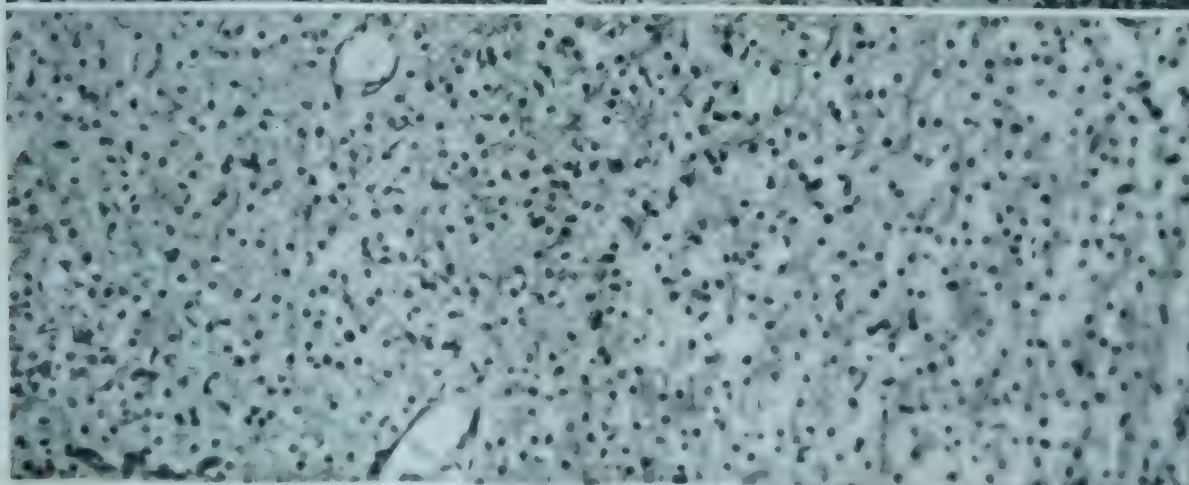
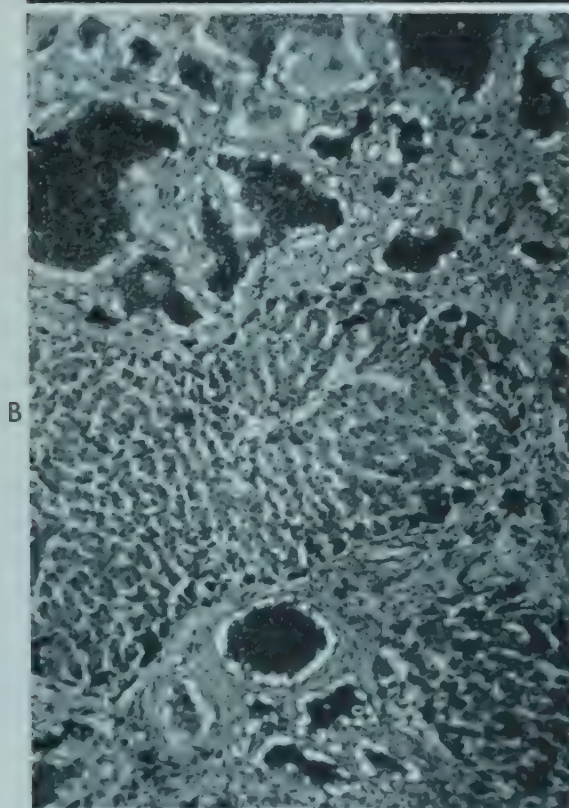
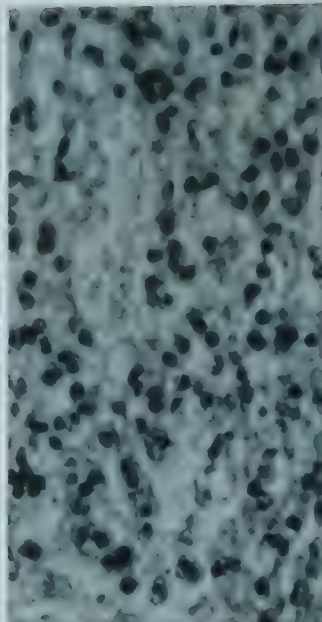
Histologic Characteristics. Solid hamartomas, aside from the hemangiomas, are focal alterations of the parenchymal architecture. In such nodules, the hepatic-cell plates are several cells thick, and the arrangement around a central vein is lost, as in the regenerative nodules. Frequently, unexplained severe fatty metamorphosis is observed, in contrast to the surrounding parenchyma. If the nodule consists only of hepatic-cell plates without bile ducts, it is an adenoma, which is very rare in man (see Hepatocellular Adenomas, later in this chapter). Usually proliferations of bile ducts and ductules and excessively formed fibrous tissue are

seen. One or the other of the elements may predominate, but often an irregular mixture is noted. Areas of inflammatory changes simulating hepatitis may be associated with hamartomas, increasing the similarity to cirrhosis [224]. Connective tissue capsules are not preformed, but large nodes compress the surrounding hepatic parenchyma and thus elicit fibrosis. From the fibrosed tissue, a capsulelike structure develops, in which remnants of the pressure-atrophied parenchyma can usually be distinguished.

Clinical Significance and Nomenclature. Small lesions usually do not receive much attention, whereas larger ones can be felt and often present a surgical problem, as exemplified by those occurring commonly in children, especially when the tumor exceeds the liver itself in size [2540]. These are anatomic curiosities [582, 1279, 1707, 1965]. Large solid hamartomas have recently been removed by surgery, the results apparently being good. The nomenclature and classification are confusing. Some authors list such lesions as adenomas, mainly of the mixed type, or benign hepatomas, while recognizing their hamartomatous nature [1707, 1965, 3498]. Others regard them as hamartomas, trying to emphasize distortion of the architecture while not denying the tumorous character [582, 1279, 2540]. Structurally they can not be differentiated from focal cirrhosis caused by unknown etiologic factors or by focal disturbances of blood supply or biliary drainage. The relatively frequent association of such lesions with malformations or other hepatic hamartomas—20 per cent of patients with these lesions have hemangiomas [224]—and the relatively frequent occurrence in children [582, 1707, 1965, 2540] strongly suggest a hamartomatous character, at least for the larger lesions.

Hemangiomas

Structural Characteristics. The most common nodule in the liver is the hemangioma, usually cavernous. Hemangiomas vary from small, barely visible nodules to large nodes weighing more than 1,000 gm. They are usually multiple, and they vary in appearance. Smaller ones are often dark red and grossly well demarcated from the surrounding tissue. Sclerosis of the hemangioma after thrombosis of the cavities accounts for complete scarring of the lesion, which then appears as a gray-white spot. When the hemangiomas become larger, there are regressive changes of fibrosis, hemorrhage and brown pigmentation appear, and a white pseudocapsule may develop, formed from



compressed and fibrosed surrounding parenchymal tissue.

Histologic Appearance. Most hemangiomas show a cavernous arrangement of thin-walled, endothelial-lined spaces filled with blood. These spaces usually communicate with the surrounding sinusoids of the liver parenchyma and rarely have their own supporting vessels. The hepatic-cell plates between the blood spaces are compressed or have disappeared. Collagenous fibers and membranes reinforce the septums between the spaces (Fig. 190B). Thrombi in various stages of organization are common. In some instances, buds lined by endothelial cells extend into the spaces. Less frequently, capillary hemangiomas, consisting of irregularly sprouting capillaries embedded in fibrous tissue (Fig. 190C), are seen, mostly in children.

Classification. The classification of these vascular tumors in general is difficult, and some authors deny the tumorous character of hemangiomas, especially in the liver [3617]. The differentiation from venectasia or capillectasia (see Peliosis, under Alterations of the Sinusoids, Chap. 56) is sometimes difficult. Small hemangiomas occur more commonly in women, especially during pregnancy, which might possibly relate them to spider nevi of hormonal origin (see Spider Nevi, under Relation between the Liver and Sex Organs, Chap. 62). The islands of budding endothelial cells and the sprouting of capillaries occasionally seen recall hemangioendotheliomas. The lack of any malignant degeneration and the frequent association with other hamartomas or malformations make these lesions appear most probably to be hamartomas, with little admixture of epithelium that is entodermal or mesodermal in origin. Hepatic telangiectasia occurs in some cases of hereditary hemorrhagic telangiectasia of Rendu-Osler-Weber, but this only partly accounts for hepatomegaly in this condition, which may also be caused by cirrhosis or congestion [3098].

Clinical Significance. Hemangiomas become a clinical problem only when they appear as a space-occupying lesion in the abdomen, with fullness or pain and gastrointestinal distress [3062]. Conspicuous compressibility of the mass and, occa-

sionally, whirring and buzzing sounds may be noted. Spontaneous hemorrhage, which can be fatal, occurs [1458]. Large, sometimes multiple hemangiomas deform the liver, producing palpatory as well as pressure phenomena, and eventually become surgical problems [1976]. Many of them have been successfully removed, and the hemangioma probably represents the most gratifying subject for partial hepatectomy. For inoperable hemangiomas, roentgen therapy has been recommended [3620].

Diffuse Hepatic Angiomatosis. In diffuse angiomatosis of the liver, confluent and nonencapsulated nodules are scattered throughout the liver (Fig. 190A). Sometimes they replace much of the liver tissue, especially in children. These lesions represent a diffuse proliferation of capillaries, usually with extensive proliferation of the endothelial cells, justifying the term "hemangioendothelioma," although less actively growing angiomatous and even cavernomatous areas are intermixed (Fig. 190D). The lesion is rare [77, 226, 1044, 1621] and has been catalogued under various names, such as "primary mesenchymal hepatomas" [1044], "hemangioendotheliomas" [226], and "multiple hemangiomas with malignant characteristics" [77]. The widespread involvement of the liver, as well as the indistinct limitation from the surrounding tissue inherent in the process of hemangiomatosis in other organs, would suggest a malignant process, particularly since similar lesions may be seen which have been considered metastases. However, such lesions are more correctly considered multicentric hemangioendotheliomas and therefore probably hamartomas. Malignant hemangioendothelial sarcomas of the liver are extremely rare and are differentiated from anaplastic carcinomas with great difficulty [1413] (see Chap. 58).

Hamartomas of the Gallbladder

Adenomyoma of the gallbladder is a rare hamartomatous lesion occurring singly, chiefly in the fundus, and presenting a honeycomb cut surface [3039]. Histologically, an admixture of glandular and muscular elements is noted, usually associated with inflammation.

FIG. 190 A. Diffuse angiomatosis of the liver. B. Cavernous angiomatosis. H&E ($\times 57$). C. Capillary angiomatosis. H&E ($\times 115$). D. Hemangioendotheliosarcoma in liver. H&E ($\times 115$). E. Hepatic adenoma nodule, consisting of hepatic cells with interspersed veins but without portal tracts, in young child. H&E ($\times 125$). (B, C, D, and E, courtesy of Dr. D. Boggs.)

BENIGN TUMORS

Hepatocellular Adenomas. If hamartomas or regenerative nodules in cirrhosis are excluded, benign hepatocellular adenomas or benign hepatomas are very rare [224, 3498]. They are encapsulated nodes of irregularly arranged hepatic-cell plates, usually several cells thick and almost devoid of ductules or ducts (Fig. 190E). No well-organized portal tracts are seen. The bile canaliculi are sometimes dilated, and bile production is noted. Adenomas grow to about fist size and occasionally are multiple.

Bile Duct Adenomas. The so-called bile duct adenomas, including the usually poorly demarcated cystadenomas [402, 3498], also belong in the group of hamartomas.

Benign Epithelial Tumors of the Biliary Tract. These tumors are primarily surgical problems and are only briefly mentioned here. Benign adenomas, papillomas, and polyps of the extrahepatic biliary ducts are rare, although they are clinically important in producing extrahepatic biliary obstruction [583, 2804]. Benign papillomas of the ampulla

may become malignant [132, 519]. Papillomas of the gallbladder are reported to be very common. They supposedly are found in 8 per cent of surgically removed gallbladders [3477]. Most of them are small, they are usually multiple, and they are found only on careful inspection of the specimen. The great majority of them, especially the smaller ones, are apparently not tumors but polyploid focal inflammatory hyperplasia, i.e., "cholecystitis glandularis proliferans" [3617]. Lipid-containing histiocytes are sometimes found in the stalks; the lesion seems to be a response to cholesterol deposition, a form of cholesterolosis. These histiocytes rarely give rise to carcinoma formation, but they can become calcified and thus lead to the formation of stones. Rarely do these papillomas become large enough to be felt at surgery or to be demonstrated roentgenologically [1774]. The larger tumors are either polyps with long stalks [3617] or flat, buttonlike adenomas consisting of cystically dilated and sometimes mucin-producing glands [3039]. Even in these larger polyps, malignant degeneration seems to be relatively rare [97, 2580].

The most important hepatic tumors are epithelial in origin. Benign epithelial tumors are not sharply defined from hamartomas, and pure forms are probably rare, while primary hepatocellular carcinoma is a clear-cut clinical entity.

EXPERIMENTAL CARCINOGENESIS

Hepatic carcinoma is a common result of experimental carcinogenesis. A discussion of the extensive amount of literature about structural and biochemical observations is beyond the scope of this book, and the problems of experimental hepatic carcinogenesis are only briefly reviewed here. Most of the studies reported concern the broader aspects of carcinogenesis in general; their applications to hepatic structure and function are limited. Many of the "hepatomas" produced seem to be regenerative nodules rather than carcinomas.

Factors in Carcinogenesis. Experimental carcinoma develops either following a stage of parenchymal necrosis or in a cirrhotic liver [930, 2489, 2494]. Prolonged hepatic regeneration, with or without experimental cirrhosis, is followed by carcinoma formation, at least in the rat. Almost every type of experimental cirrhosis in rats seems eventually to be followed by carcinoma. Whether a specific carcinogenetic effect is present or whether cirrhosis with prolonged regeneration is responsible can not be decided. The latter possibility has to be entertained, at least for the hepatic neoplasms produced in rats by prolonged choline deficiency, ethionine administration, or carbon tetrachloride intoxication. This is in contrast to the spontaneous hepatic carcinomas in aged mice in which the liver is not cirrhotic.

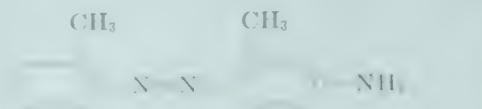
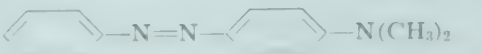








CHEMICAL CARCINOGENS. The most commonly used chemical carcinogens are the azo dyes. The

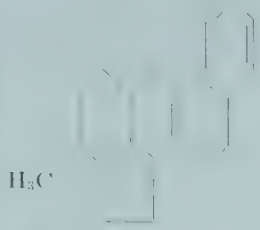
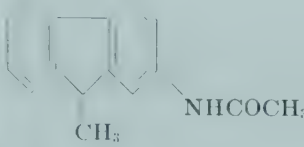
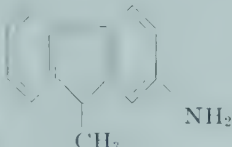
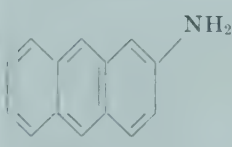
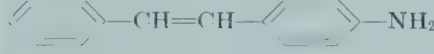
first one used was ortho-aminoazotoluene in rats and in mice. Rabbits or dogs are refractory. *P*-dimethylaminoazobenzene, or butter yellow, has subsequently been given most widely to rats for the production of hepatic carcinoma [1763]. Many additional related dyes and carcinogenic hydrocarbons, such as 2-acetylaminofluorene and 4-aminostilbene, have been used [240] (Table 61). Administration of ethyl urethane [1623] or thiourea, thioacetamide, or DDT [1028] also produces hepatic neoplasms [1623]. The cirrhosis resulting from carbon tetrachloride [891], from selenium [2422], or from senecio [644] may be followed by formation of hepatic carcinoma. Exposure to radioactive material occasionally leads to tumor formation [240, 3395]. Injected plutonium produces lesions similar to those produced by the carcinogenic azo dyes, but full-fledged carcinomas have not been observed [2031]. As already mentioned, all types of protracted hepatic injury appear to result, at least in rats, in carcinoma formation, which implies that the reaction is not specific for the group of substances usually designated as carcinogens.

NUTRITIONAL FACTORS. Hepatic neoplasms develop after prolonged choline deficiency, an illustration of nutritional factors resulting in cancer formation [655, 2903]. Prolonged feeding of ethionine, a methionine antagonist, also produces hepatic tumors [2635].

AGE, SEX, AND GENETIC FACTORS. In several strains of mice, inbreeding increases the tendencies for carcinoma formation, indicating a genetic factor [444, 1233]. Female mice seem to be more susceptible to aminoazotoluene, as well as to butter yellow, suggesting sex differences in tumor formation [240]. Aging is apparently also a factor, as shown in the incidence of spontaneous hepatic

Table 61 Hepatoma-inducing Chemicals. Their Formulas, the Animals in Which They Have Been Tried, and Their Ability to Produce Cirrhosis

Name	Formula	Animal	Cirrhosis	Reference
Azo compounds:				
Ortho-aminoazotoluene.....		Rat Mouse	No No	240
Para-dimethylaminoazobenzene...		Rat	Yes	1766 344
Para-aminoazobenzene.....		Rat	Little	1772
m'-methyl-p-dimethylaminoazo- benzene		Rat	Most severe Most potent	1161 2292
p-monomethylaminoazobenzene...		Rat	Yes	1161 2292
2:2'-azonaphthalene.....		Mouse	Very little	645
2:2'-diamino-1:1'-naphthyl.....		Mouse	Very little	645
Carcinogenic hydrocarbons:				
3:4:5:6-dibenzcarbazole.....		Mouse	Slight	240
1:2:5:6-dibenzanthracene		Mouse	No	72
1:2-benzanthracene		Rat	Yes	358

Name	Formula	Animal	Cirrhosis	References
Methyl cholanthrene		Mouse	No	3254
2-acetylaminofluorene		Rat Mouse Cat	No Yes No No	3625 3090 98 273
2-aminofluorene		Rat Mouse	No No	273 3618
2-anthramine		Mouse	No	240
4-aminostilbene		Rat	No	1333

cell carcinomas in very old dogs [2376] and other animals [3617].

OTHER TUMOR-INDUCING FACTORS. Sarcomas of the hepatic connective tissue develop following trypan blue injections [1172] or feeding of ova of the cat tapeworm. Endogenous carcinogenic chemicals have been demonstrated in man, particularly in liver tissue and bile, independent of race, age, sex, or the presence or absence of cirrhosis [3195].

ACCELERATING AND INHIBITING FACTORS. Many factors inhibit or accelerate carcinoma formation [241]. In the presence of rapid regeneration, as induced by partial hepatectomy, hepatic tumors develop more quickly [1190]. Fat and rice or rice extracts accelerate formation of azo dye-induced tumors [2489]. Riboflavin and yeast, as well as its extracts, prevent carcinoma formation, because riboflavin competes with the azo dye for the protein prosthetic group of coenzymes [2293, 3260]. The amount of riboflavin in the liver therefore can be the decisive factor in carcinogenesis [2289]. The preventive effect of liver and liver extract probably results from the same factors. Protein and amino acids, which prevent hepatic necrosis

and cirrhosis (see Nutritional Deficiencies, Chap. 50), retard formation of neoplasms. Casein, cystine, and choline protect against the carcinogenic effects of butter yellow [1321], although this was denied [3584]. Methionine, supplemented with casein or high-casein diets, retards the development of carcinomas produced by azo dyes [1280], possibly by increasing the riboflavin content of the liver. Low-protein diets or low concentrations of sulfur amino acids in the diet reduce the incidence of spontaneous hepatic carcinoma [3074]. The addition of 0.25 per cent copper to the diet exerts an inhibitory effect on tumor formation, the mechanism of which is unknown [2553].

Histology of Experimental Hepatic Neoplasms.
TYPES OF CELLS. The cells of the neoplasms induced in experimental animals resemble hepatic cells, ductal cells, or ductular cells. In most experimental neoplasms, all types of cells are found if a large enough sample is examined. Moreover cellular structures are frequently seen which are transitional forms. Differentiation of hepatic cells into ductular and ductal cells has to be assumed, in keeping with embryologic experiences and as sug-

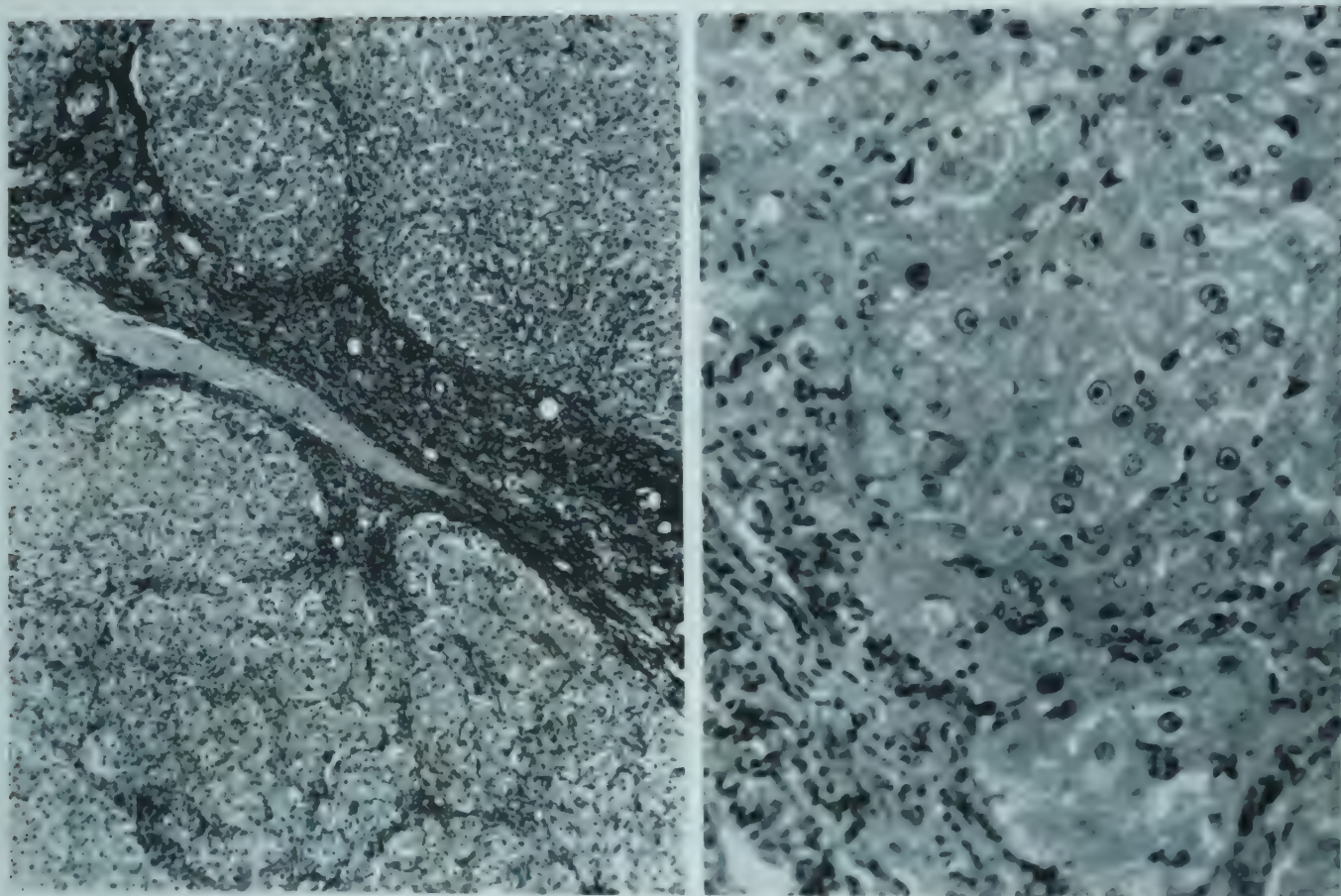


FIG. 191 Liver of rat which had been alternating on an 0.5 per cent ethionine diet and on a normal diet for 409 days (*in toto* 268 days on the ethionine diet). *Upper*. Large nodule protruding over the surface of a cirrhotic liver. H&E ($\times 4$). *Lower left*. Hyperplastic nodules of various sizes embedded into dense connective tissue containing inflammatory exudate, proliferated bile ducts, ductular cells, and a few scattered hepatic cells. The left lower half represents the protruding nodule, composed of large hepatic cells arranged in several-cell-thick plates and traversed by a few connective trabeculae. H&E ($\times 70$). *Lower right*. Higher magnification of lower half of picture to the left, showing large hepatic cells (with large nuclei and nucleoli) arranged in several-cell-thick plates without intervening stroma. H&E ($\times 265$). (Popper, H., and Bruce, C.; *J.Nat.Cancer Inst.*, 15:1597, 1955.)

gested for primary hepatic carcinoma (see Human Primary Hepatic Carcinoma, later in this chapter). Experimental hepatic tumors receive their afferent blood supply almost entirely from the hepatic artery [388]. This also suggests an origin from multipotent cells near the portal tracts, similar to that of human carcinoma. Multicentric origin of

hepatic neoplasms seems to be the rule, at least in experimentally induced tumors.

DEFINITION OF EXPERIMENTAL HEPATIC NEOPLASM. The division between hyperplasia and neoplasia in the liver is sometimes difficult. Cytologic characteristics of neoplasia are of limited value. Differentiation between actively regenerating nod-

ules, especially in cirrhosis, and benign tumors is virtually impossible. To avoid confusion in nomenclature, the notion of a benign hepatocellular or bile duct adenoma in experimental animals is best dismissed. In this sense, only hepatic carcinomas are accepted as hepatic neoplasms. Invasion of vessels or of the surrounding tissue, tumor embolism, distant metastases, or successful transplantation serve as an index of malignancy. Nodules larger than 1 cm or necrosis of the central portion is suggestive but not diagnostic of carcinoma. Many tumors in experimental animals become questionable, and the term "hepatoma" itself is meaningless in the light of these strict definitions. Lesions such as cholangiofibrosis or hepatic nodules which are found in animals on carcinogenic regimens before unquestionable carcinomas appear are thus not considered neoplastic. They are not transplantable and regress upon discontinuing the carcinogenic stimulus. Two patterns of cancer development are seen which are common to different types of carcinogenic regimens. Only the final stage is acceptable as carcinoma.

HEPATOCELLULAR NODULE. In various types of intoxication, for instance after prolonged administration of ethionine [2635] or thioacetamide [1028], nodular thickening of the hepatic-cell plates develops, usually in areas of hepatic parenchyma subjected to extensive focal necrosis and regeneration, as well as diffuse mesenchymal reaction and proliferation of cells derived from bile ductules. In these nodules the hepatic cells are larger (Fig. 191, lower right). The basophilia of their cytoplasm is increased and diffusely dispersed [2604]. Their nuclei are larger than those in the surrounding parenchyma, and multiple nucleoli are noted. The mesenchymal and ductular-cell reaction is absent, and the reticular framework can be impregnated only poorly. These very actively regenerating nodules increase in size until they seem to bulge over the surface of the liver (Fig. 191, upper) and compress the surrounding parenchyma (Fig. 191, lower left). Their growth sometimes persists after subsidence of the hepatotoxic stimulus and the return of the surrounding parenchyma to normal. Up to this stage, carcinoma formation is not present. Eventually, syncytial cords or adenomatous structures develop [892], and the signs of malignancy appear (Fig. 193, upper right).

BILE DUCTAL AND DUCTULAR NODULE. In various intoxications, such as that produced by butter yellow [892, 1171] or ethionine [2635], the epithelial cells of periportal ductules and septal bile

ducts in many portal tracts multiply, resulting in a piling-up of the cells, the presence of mitosis, and sprouting of the ducts (Fig. 192B). This epithelial hyperplasia may involve only parts of the circumference of the ducts. The lumen becomes dilated and filled with mucus (Fig. 192D). The basement membrane is thickened, and the connective tissue around the sprouts is increased. Nodules form, connected with the portal tracts, which become eventually grossly visible as large white nodes (Fig. 192A). The lesion has been called "cholangioma," but it is better designated as "cholangiofibrosis" [892]. It regresses or disappears after cessation of the carcinogenic stimulus and becomes transformed into scar tissue, with a few remaining epithelial ducts, which may show mitosis. A malignant lesion develops if the epithelial lining shows papillary excrescences, and it breaks through the basement membrane (Fig. 194, right). At that stage necrosis is usually present, in addition to the other characteristics of malignancy.

HEPATIC CARCINOMA FROM BUTTER YELLOW. The lesions produced by butter yellow [891, 892, 2489] or by aminoazotoluene [72] represent the prototypes of experimental hepatic carcinoma. Butter yellow initially produces severe degenerative changes, with central or focal necrosis, fatty metamorphosis, and excessive regeneration [2495] (Fig. 132C), associated with disappearance of basophilia and chromatolysis [2495]. The lesion is associated with severe portal and periportal, histiocytic, and lymphocytic infiltration and with proliferation of the ductules. Alteration of the ductules has been considered the initial process in carcinoma formation [2489]. Regeneration and hyperplasia of hepatocellular ductal and ductular elements produce great variations of the appearance of the liver in animals of the same experimental series [2489]. Hepatocellular nodules form (Fig. 193, upper left), which may show transition into a trabecular type of carcinoma. Some of the trabeculae have central lumens resembling ductules (Fig. 193, upper right). The cells are undifferentiated, and giant cells can be noted (Fig. 193, lower). The ductular transformation via cholangiofibrosis leads to formation of unquestionable adenocarcinoma (Fig. 194, left) and papillary cystadenomacarcinoma (Fig. 194, right).

Tissue Changes Associated with Tumor Formation. In principle hepatocellular carcinomas behave biochemically like peripheral tissue rather than liver. Alterations in hydrogen ion concentra-

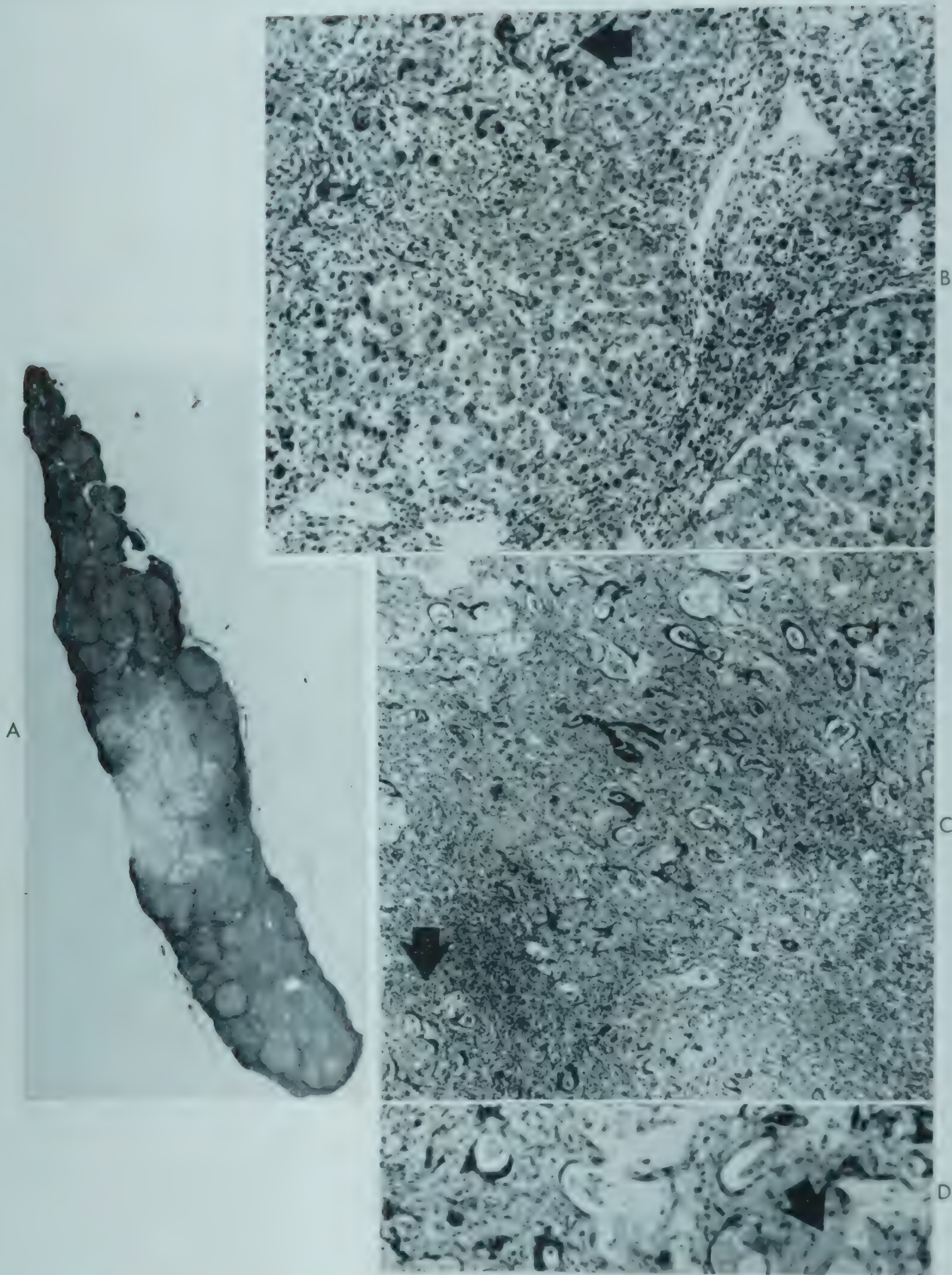


FIG. 192 A and B. Liver of rat which had been alternating an 0.5 per cent ethionine diet and a normal diet for 412 days (*in toto* 208 days on the ethionine diet). A. Nodule consisting of dense connective tissue with isolated, hardly recognizable strands of proliferated bile ducts. H&E ($\times 4$). B. Close up of border of fibrotic nodule, revealing irregularly arranged regenerating hepatic cells surrounded by ductular cells and reticuloendothelial cells. In the connective tissue trabeculae, proliferated bile ducts and inflammatory exudate are noted. In the right upper corner (arrow), irregularly arranged bile ducts with actively proliferating epithelium embedded in dense connective tissue are noted; cholangiofibrosis. H&E ($\times 75$).

C and D. Liver of rat which had been alternating an 0.5 per cent ethionine diet and a normal

tion [1674], enzymes [1275], cytoplasmic basophilic substances [2489, 2604, 2860], nuclear and nucleolar desoxypentose nucleic acids [708, 3239], and nuclear lipids, phospholipids, and cholesterol [766] have been reported, just to cite a few examples. The hepatic parenchyma not involved by carcinoma is also chemically altered. In the presence of nonhepatic tumors, changes in hepatic enzymes have been observed [1276] and can be demonstrated in parabiotic animals [2084]. In parabiotic partners of tumor-bearing animals, the mitochondria and the hepatic cells change in size and shape [80]. The number of mitoses in the liver is increased.

HUMAN PRIMARY HEPATIC CARCINOMA

The primary hepatic carcinoma has attracted the interest of clinicians, pathologists, and experimentalists out of proportion to its incidence in the Temperate Zone and the chance for cure. This is the result of several factors: (1) the ease of experimental production of hepatic cancers and the possibility of theories of carcinogenesis resulting from these observations; (2) the great geographical variation in its incidence, since it seems to be the most frequent cancer in the Tropical Zone; (3) the high incidence in cirrhotic persons; (4) its occurrence as a congenital tumor early in life. Many classifications of primary hepatic carcinoma exist, based on varying views about its histogenesis.

Histogenesis of Primary Hepatic Carcinoma. HEPATOCELLULAR VS. CHOLANGIOCELLULAR CARCINOMA. All authors agree upon a division into hepatocellular carcinoma, derived from the polygonal hepatic cells, and bile duct carcinoma, often called "cholangiocarcinoma," derived from the epithelium of the bile ducts [240, 888, 1562, 3498]. A different dividing line between these two types is drawn by various observers. However, in the same liver and even in the same cancer nodule, hepatocellular epithelium as well as ductular and ductal epithelium are often noted. The larger the sample, the more common is the mixture. Moreover, one often sees transitional structures which could originate from either hepatic cells or ductular cells. This makes it difficult to designate tumors com-

posed mainly of derivatives of hepatic cells as hepatocellular carcinoma, and those composed mainly of derivatives of ductal and ductular structures as cholangiocellular carcinoma. In addition, the biologic characteristics of primary hepatic carcinoma, viz., tendency to invasion of the portal vein branches, male predominance, frequent coexistence with cirrhosis, bile formation, tendency to multicentric origin, and high incidence in young adults in the tropics, are found with equal or nearly equal frequency in so-called hepatocellular carcinoma and in carcinomas with predominance of cholangiomatous features. In contrast, they are not found in the relatively rare tumors composed entirely of high columnar bile duct epithelium and apparently derived from the epithelium of large intrahepatic bile ducts. These tumors are similar in their morphologic appearance to carcinomas of the extrahepatic bile ducts.

ORIGIN OF DUCTULAR CARCINOMA. Embryologic observations indicate that ductules and ducts are derived from the hepatic cells, possibly as a result of organizing influences from the mesenchyme. Such developmental potentialities can also be ascribed to hepatic carcinoma cells. Therefore all carcinomas revealing some hepatic cells can be considered as a single entity, regardless of the presence or even predominance of cholangiomatous features. Experimental studies strongly support the idea that hepatocellular and ductular cell tumors are a unit [892, 1766].

SITE OF CARCINOMA FORMATION. Primary hepatic carcinoma has been said to originate from hypertrophic hepatic-cell plates which show nodular hyperplasia, adenoma formation, and finally carcinomatous change. Ductular alterations with pericholangiolitis and cholangiofibrosis precede carcinoma in rats treated with butter yellow [2489]. The tissue of primary hepatic carcinoma differs from normal hepatic parenchyma and resembles portal tracts, in that its blood is supplied by the hepatic artery and it is drained by the portal vein [388]. This supports the idea that carcinoma develops from a multipotent cell [240] in the limiting plate, a cell which can differentiate into hepatic cells as well as ductular and ductal epithelium.

diet for 134 days, then was on a normal diet for 529 days, and subsequently was again on the alternating diet for 166 days. C. Border zone of nodule revealing cholangiofibrosis (arrow), and, in the central portion, irregularly arranged glandular structures in a fibrosing stroma. H&E ($\times 57$). D. Mucinous material in the lumen of the glandular structures. The epithelial lining is in places missing, and the mucinous material lies free in the connective tissue (arrow). H&E ($\times 115$). (Popper, H., and Bruce, C.: *J.Nat.Cancer Inst.* 15:1597, 1955.)

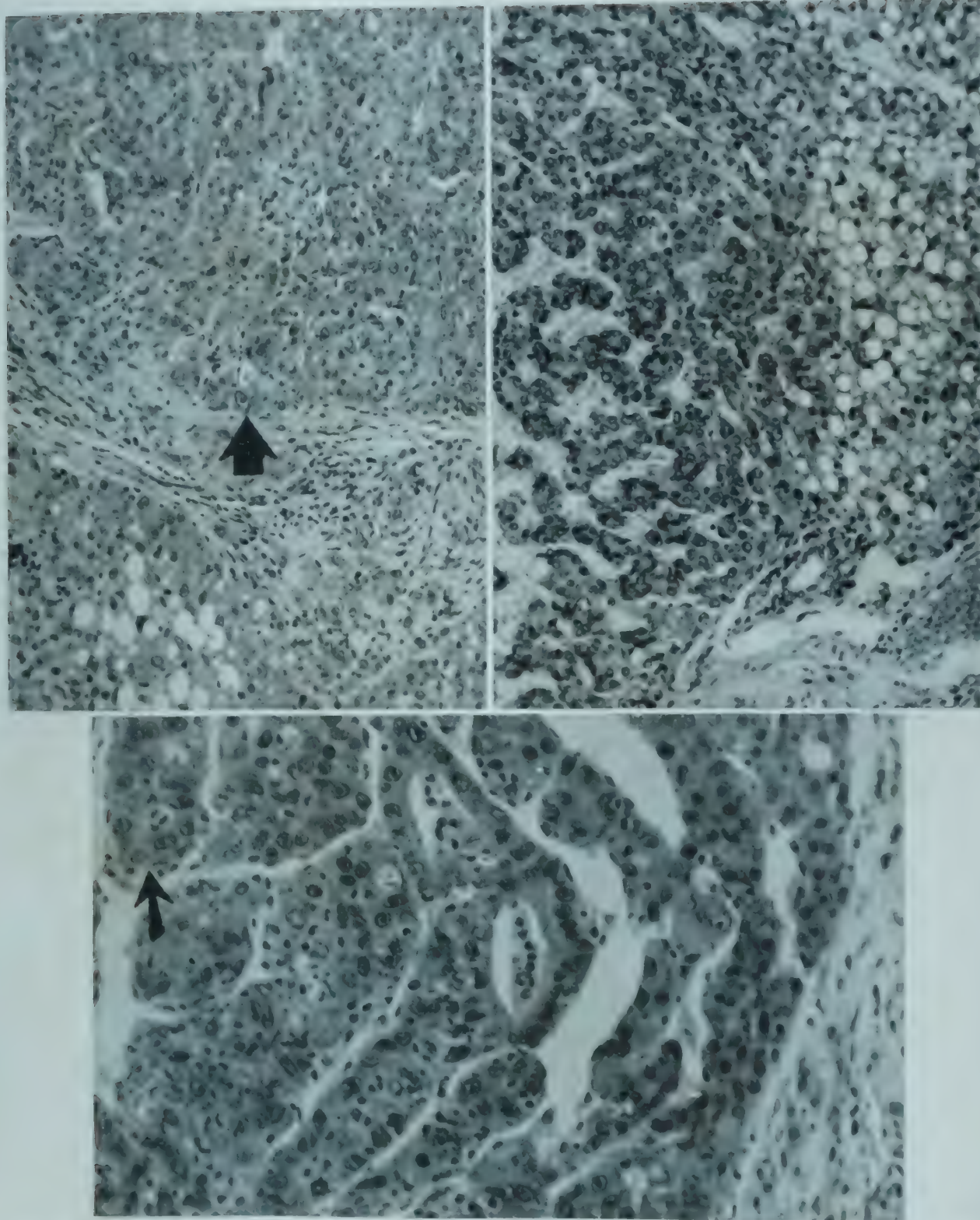


FIG. 193 Rats after prolonged administration of butter yellow. *Upper left.* Hepatocellular nodule (arrow) in a fatty liver. H&E ($\times 150$). *Upper right.* Trabecular hepatocellular carcinoma in fatty liver. H&E ($\times 150$). *Lower.* Trabecular hepatocellular carcinoma with giant cells and focal transition into ductules (arrow) ($\times 290$). (Material of Drs. Julius White and Jesse Edwards, obtained through the courtesy of Dr. Thelma B. Dunn, Laboratory of Pathology, National Cancer Institute.)

PATHWAYS OF CARCINOMA FORMATION. Three-dimensional analysis of primary hepatic carcinoma [910] indicates three morphologic pathways of carcinoma, resembling stages of embryonal devel-

opment, which are not necessarily found in man but in other vertebrates:

1. Globular and sheetlike masses of carcinoma cells resembling hepatic cells develop, without

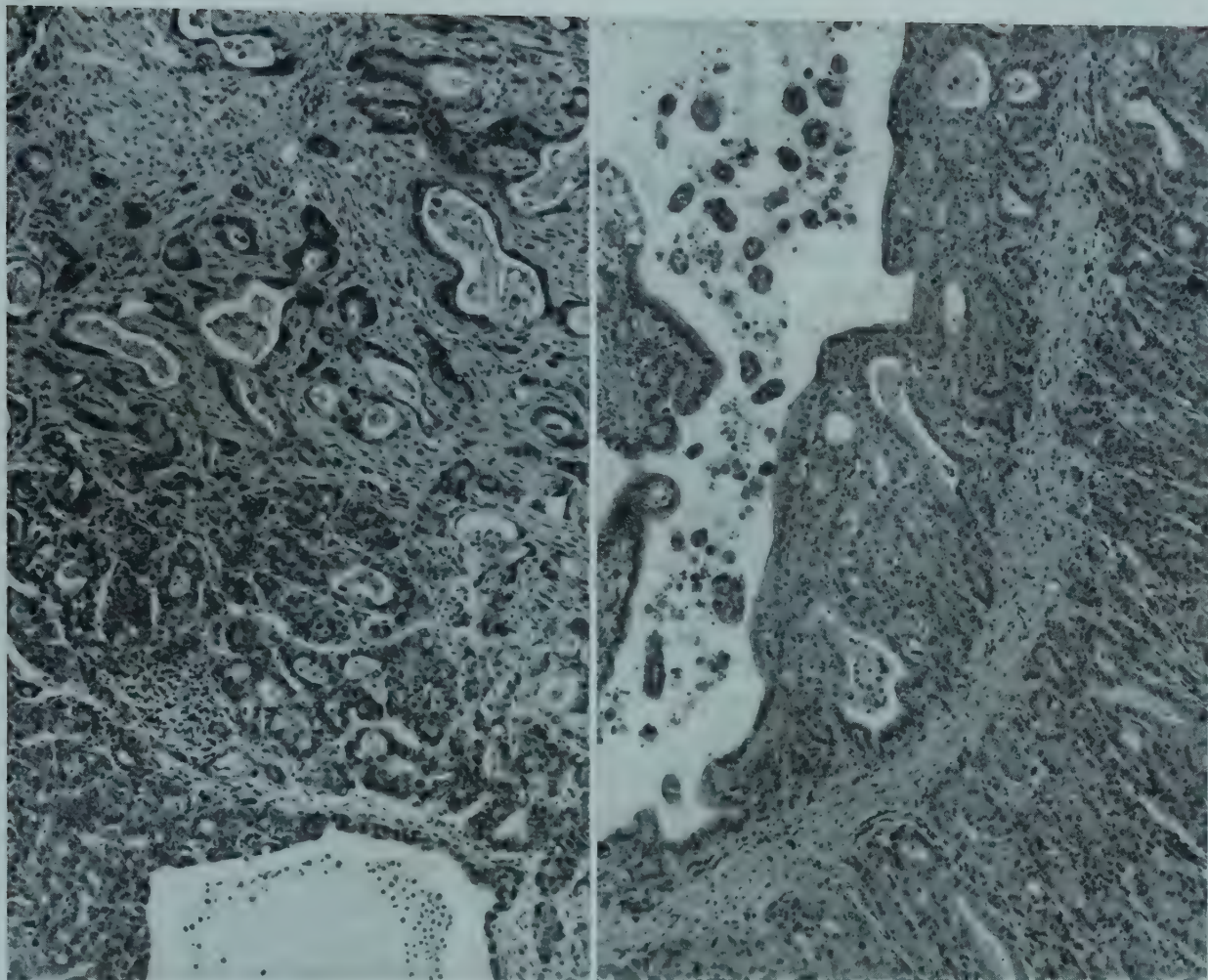


FIG. 194 Rats after prolonged administration of butter yellow. *Left.* Cholangiofibrosis in upper part and ductular adenocarcinoma in lower part of picture. H&E ($\times 85$). *Right.* Papillary cystadenocarcinoma ($\times 90$). (Material of Drs. Julius White and Jesse Edwards, obtained through the courtesy of Dr. Thelma B. Dunn, Laboratory of Pathology, National Cancer Institute.)

internal stroma, surrounded by the original sinusoidal endothelium and corresponding to the trabecular type of carcinoma.

2. Two-cell-thick plates of cancer cells surrounded by excessive connective tissue degenerate into a flat "wallwork," which in histologic sections resembles ductules the lumina of which do not contain bile.

3. Cancer cells in short tubules filled with bile, not connected with each other, develop within the plates and resemble either acinar structures or ductules in histologic sections, thereby justifying the term "adenocarcinoma."

UNICENTRIC OR MULTICENTRIC ORIGIN. Multiple cancers develop in the liver of experimental animals. Whether primary cancer in the human liver develops from one or several sites remains unsettled. Single, small cancers found incidentally speak for unicentric origin. Most recent observers believe that all human cancers are unicentric and that

transitions of normal cells into malignant ones are an illusion created by invasion of the original connective tissue framework by the cancer [240, 888]. Multiple nodules of equal size in the same liver are explained as venous metastases. However, in three-dimensional analyses, at least in the presence of cirrhosis, multiple transitions of normal one-cell-thick plates into two-cell-thick plates are seen, the cells of the latter exhibiting cytologic criteria of malignancy [910]. Therefore, carcinomas developing in cirrhotic livers usually seem to be multicentric, while in noncirrhotic livers they are unicentric.

NOMENCLATURE. In the following description, primary hepatic carcinoma is used for both hepatocellular carcinoma and cholangiocarcinoma with the presence of some hepatic cells, in contrast to bile duct carcinoma, apparently derived from large bile ducts. Where possible, this viewpoint is applied to evaluation of published observations.

Incidence. The incidence of primary hepatic carcinoma depends on age, sex, geographical distribution, and preexisting liver disorders, especially cirrhosis.

AGE AND SEX INCIDENCE. These carcinomas occur in all age groups, but in the Temperate Zone the incidence increases sharply in older age groups [888]. In Europe or the United States it appears mainly in the fifth or sixth decade [888, 1562, 2816]. In nonwhite Americans it occurs in younger age groups. The cases in infancy and childhood seem to belong to a different group, possibly of congenital origin, and are discussed independently (see Primary Hepatic Carcinoma in Childhood and Infancy, later in this chapter). In the tropics or near tropical areas, primary hepatic carcinoma is common in young adults, for instance among the South African Bantu or in the Philippine Islands [240, 888]. In general, if the incidence is high in any location, it often occurs in young adults [888]. This carcinoma is more common in males of all ethnic groups [240, 888, 2090, 2816], possibly because of the greater incidence of cirrhosis in men. It also occurs more often in diabetic persons, again possibly because of the increased incidence of cirrhosis [888].

GEOGRAPHIC DISTRIBUTION. In the United States and Europe, primary hepatic carcinoma is found in less than 0.3 per cent of all autopsies and accounts for less than 2.5 per cent of all carcinomas. In the tropics, the incidence is much higher; this type of carcinoma is found in almost 1 per cent of all autopsies in Orientals, and the percentage is even higher in the Bantu [240, 888]. The increased incidence in the Tropical Zone is also reflected in the high percentage of primary hepatic carcinomas in comparison to carcinomas of other organs. It represents about 14 per cent of all carcinomas in tropical areas of the Orient or in Central Africa, while in the Bantu it rises to 50 per cent [240].

RACIAL FACTORS. Significant racial differences are seen in the same area. In South Africa, the incidence is more than five times higher among South African natives than among East Coast natives, all working together in the same mines [240]. In the American Negro the incidence is only insignificantly higher than in the Caucasian [2090, 3196], being considerably less than in the same race in Africa. In contrast, the incidence in the American Oriental is higher than in the same race in Asia [240, 888, 2816, 3196, 3253, 3598].

ENVIRONMENTAL FACTORS. The geographical studies, for instance on the Negro race, implicate environment instead of heredity [240, 888, 3196], environment changing upon migration from Africa to America. In the more recent Oriental immigrants the incidence remains high for some time, possibly because of an environmental factor acquired in childhood. Parasitic infections have been considered an important environmental factor in Oriental immigrants [240, 888, 1620, 2775], especially schistosomiasis or clonorchiasis. The parasitic infestation possibly is coincidental and not the cause of the cancer [3196], except in infestation of Orientals with *Clonorchis sinensis*, which produces a morphologically peculiar type of cancer [1410]. Around the infested bile ducts, which often also contain calculi, irregular ductular proliferation develops and progresses to carcinoma. Malnutrition is a far more important environmental factor, although the character of the malnutrition is not established [3196]. The influence of malnutrition is reflected in the frequent coincidence of cirrhosis and primary hepatic carcinoma.

Relation of Carcinoma to Cirrhosis. Cirrhosis is associated with primary carcinomas of the liver in from 34 to 82 per cent of cases [240, 628, 888, 1353, 1562, 2090, 3253]. The reported incidence of cirrhosis is considerably higher in hepatocellular carcinoma than in cholangiocarcinoma. For instance, in one series cirrhosis was present in over 80 per cent of hepatocellular carcinomas and only in 23 per cent of bile duct carcinomas [888]. In other series, the figures were 75 and 18.2 per cent [1562] and 58.9 and 21 per cent [2090], respectively. Since hepatic carcinomas with predominantly cholangiomatous features are often included with bile duct carcinomas [240], the difference may be even greater.

Some investigators question a diagnosis of primary hepatocellular carcinoma without cirrhosis [674], although many primary hepatic carcinomas develop without cirrhosis in man [49, 302], as well as in animals with experimentally induced or spontaneous carcinomas. In Austria 60 per cent and in Malaya 50 per cent of primary hepatic carcinomas develop in the absence of cirrhosis. Carcinoma developing in the absence of cirrhosis has been considered to be the result of focal cirrhosis or hamartomas [224] (see Hepatic Hamartomas, Chap. 57).

Laennec's cirrhosis, as a result of malnutrition or alcoholism, is the type most frequently associated

ated with carcinoma [888]. This explains the high incidence of alcoholic persons among patients with carcinoma of the liver. The incidence of carcinoma in Laennec's cirrhosis is about 5 per cent, while in less-advanced fatty cirrhosis, at least in our material, it is only a tenth of this percentage. Association of carcinoma with postnecrotic cirrhosis has also been mentioned [3037, 3196]. Cirrhosis in hemochromatosis is more often associated with primary hepatic carcinoma, occurring in 7 to 20 per cent of cases [233, 888, 3492] (see Carcinoma Formation, under Hemochromatosis, Chap. 53), possibly because of the long duration of the cirrhosis. Ductular cells appear to be more common in carcinomas found with hemochromatosis.

The incidence of hepatocellular carcinoma in white patients with cirrhosis is 2.8 to 6.2 per cent [233, 240, 1353, 2090], or at least twenty times more common than in patients without cirrhosis [2090]. In women with Laennec's cirrhosis, the incidence is low [888]. Since long-standing cirrhosis is apparently required for carcinoma formation, an increased incidence of cancer in cirrhosis is predicted because of the longer life expectancy resulting from improved treatment [240].

In some areas of Asia and Africa, both cirrhosis and primary hepatic carcinoma have a very high incidence [408], but they do not necessarily run parallel. For instance, in India a high incidence of cirrhosis, but not of cancer, is found [3196]. The same holds true for Jamaica.

Despite the large amount of material collected, the relation between cirrhosis and primary hepatic carcinoma is still unsettled. Both are possibly produced independently by the same agent, although experiences with experimental animals suggest that the cirrhotic changes predispose to cancer formation. This process is more rapid in experimental animals, while in man, so many years are required that most persons with cirrhosis do not live long enough to develop hepatic cancer.

Clinical Manifestations. The clinical manifestations of hepatocellular carcinoma are notoriously confusing and misleading. A series of thorough clinical studies emphasizes their diverse and protean natures [628, 1121, 1531, 2816, 3145, 3253]. Only in recent years has the carcinoma been commonly diagnosed during life. Various attempts have been made to subdivide the clinical picture into different types [240, 888]; the first two are more common, while the remaining four are considered atypical [240]:

1. Typical cancer with manifestations of a rapidly progressive malignant process, characterized by asthenia, abdominal pain, dyspnea, cachexia, emaciation, hepatomegaly, nodularity, and tenderness of the liver, often complicated by jaundice and ascites.

2. A sudden and otherwise unexplained alteration of the clinical course of cirrhosis, with a rapid downhill progression, usually without jaundice but with splenomegaly, ascites, pain, tenderness, and sudden enlargement of the liver, with increased nodularity, the appearance of a venous hum, or bruit, over the liver, and, often, deterioration of the hepatic function.

3. Acute abdominal distress characterized by the sudden appearance of alarming symptoms caused by hemorrhage from a carcinomatous nodule or erosion of a vessel.

4. Acute febrile illness characterized by sudden and severe pain and tenderness over the liver accompanied by severe systemic manifestations suggestive of toxemia.

5. Symptoms of metastases to other sites, such as bones [499, 628], lungs with pleural effusion [464], or even the brain.

6. Occult cancer, complicating another disease, incidentally found at operation, by biopsy, or even at autopsy.

Taking all groups together, the following clinical manifestations occur in order of decreasing incidence: a rapidly growing tender abdominal mass with no primary tumor demonstrable anywhere else, abdominal pain, abdominal distention, weight loss, fever, anorexia, nausea and vomiting, anemia, jaundice, ascites, and edema. Jaundice results from the associated cirrhotic process, from toxic damage to the liver, or from compression of the invaded bile ducts. The gastrointestinal symptoms include constipation or hematemesis from esophageal varices, which have been reported to occur in 16.6 per cent of primary hepatic carcinomas [888]. Unexplained hypoglycemia occurs, which is sometimes of diagnostic significance [3325]. The venous bruit, or hum, heard over the liver is possibly caused by the increased arterial blood flow, which may be shunted into veins directly [240]. Symptoms mainly caused by the cancer itself include pain, weight loss, asthenia, and severe anemia; those resulting from cirrhosis are ascites, edema, and splenomegaly; and those produced by both are hepatomegaly, hepatic tenderness, and jaundice.

Laboratory Findings. The laboratory findings reflect both the cirrhosis and the malignant tumor. Leukocytosis is fairly frequent, and thrombocytes are sometimes increased. The results of flocculation tests are abnormal, cholesterol esters and prothrombin concentration are reduced, hyperbilirubinemia is frequent, and serum gamma globulin may often be increased [888, 1121]. These results are of little help in establishing the diagnosis. Two findings are suggestive of primary carcinoma, but their absence does not exclude this diagnosis. One is elevation of serum-alkaline phosphatase activity out of proportion to the degree of jaundice [1315]. This happens occasionally in cirrhosis, even in the absence of jaundice [2640], but should arouse suspicion of primary carcinoma. The other is the increase of serum mucoprotein to high levels during the course of cirrhosis, since the levels are low in uncomplicated cirrhosis even with jaundice [1272]. Occasionally the laboratory findings of cholestasis without severe jaundice, namely increased alkaline phosphatase activity and high total cholesterol, are encountered. These increases are caused by obstruction of one branch of the hepatic duct by the carcinoma.

Structural Alterations

Macroscopic Appearance. The liver that harbors a primary hepatic carcinoma is enlarged and contains nodes differing in structure, color, and consistency from the surrounding normal or cirrhotic parenchyma. The arrangement of these nodes is the basis of various classifications [240, 888, 1562, 2090]: (1) massive, in which one large node predominates, although it may be surrounded by many small daughter nodules (Fig. 195, top); (2) nodular, in which nodules of varying sizes are distributed throughout the parenchyma (Fig. 195, center); (3) diffuse, almost always associated with cirrhosis in which grossly the tumor tissue can not easily be differentiated from uninvolved tissue (Fig. 195, bottom). The cirrhotic transformation is usually far advanced (Fig. 196A). The tumor nodules show various degenerative changes, such as hemorrhage or necrosis. They bulge on the surface. Intact nodules usually have a homogeneous, slightly granular appearance. Some nodules exhibit a very coarse and somewhat distorted lobular architecture. This is caused by carcinoma growth in the preformed connective tissue framework, thus preserving shadows of the former structures. The color of the nodules varies

in the same liver, and some are green or brown because of bile formation. The surrounding tissue is compressed, and tumor thrombi, sometimes mixed with fibrin thrombi, are found in portal vein branches, only rarely in hepatic vein branches, in the main stem of the portal vein, and exceptionally in the inferior vena cava (Fig. 199C). Areas of hemorrhagic infarction are found throughout the liver. Adhesions to the diaphragm and surrounding structures of the abdominal cavity form and become firm as a result of carcinomatous peritonitis. In general, the right lobe is more frequently involved than the left lobe, probably owing to its greater mass. Peculiar variations of distribution include rare pedunculated tumors.

Histologic Changes. The basic cell type is polygonal with a distinct cell border, a loose cytoplasm abundant in basophilic granules, one or two large vesicular nuclei, and very large nucleoli [240, 888, 1562, 3372, 3502] (Fig. 198B). These cells are arranged in plates several cells thick around conspicuous bile canaliculi, which are often dilated (Fig. 198B) and may contain bile plugs. Around these bile canaliculi the cells are commonly elongated and grouped in an alveolar fashion (Figs. 197, upper left, 198A, B). In such areas the differentiation from ductular structures is difficult. The plates may contain several bile canaliculi and are irregularly arranged, varying in width and very frequently connecting with each other (Fig. 197, upper right). The cells on the periphery of thicker plates are smaller and cuboidal, as in a limiting plate. The basic structure thus appears trabecular, a term frequently applied in the description of hepatocellular carcinoma. The plates are surrounded by sinusoids and an argentaffin framework traversing the perisinusoidal space, which is usually recognized because of the presence of protein deposits in it. In some areas this reticulum becomes collagenized, especially if it appears to be under pressure. This often occurs on the periphery of the nodes, thus producing a pseudocapsule. The sinusoidal endothelial cells resemble Kupffer cells and often contain engulfed material and fat droplets. This basic arrangement simulates the structure of the normal embryonal liver. It is most clearly seen where the tumor grows into veins, as occurs typically in primary hepatic carcinoma.

VARIATIONS. Attempts at histologic subclassifications are based on variations in the basic picture. Many variations are found in the same liver, including:

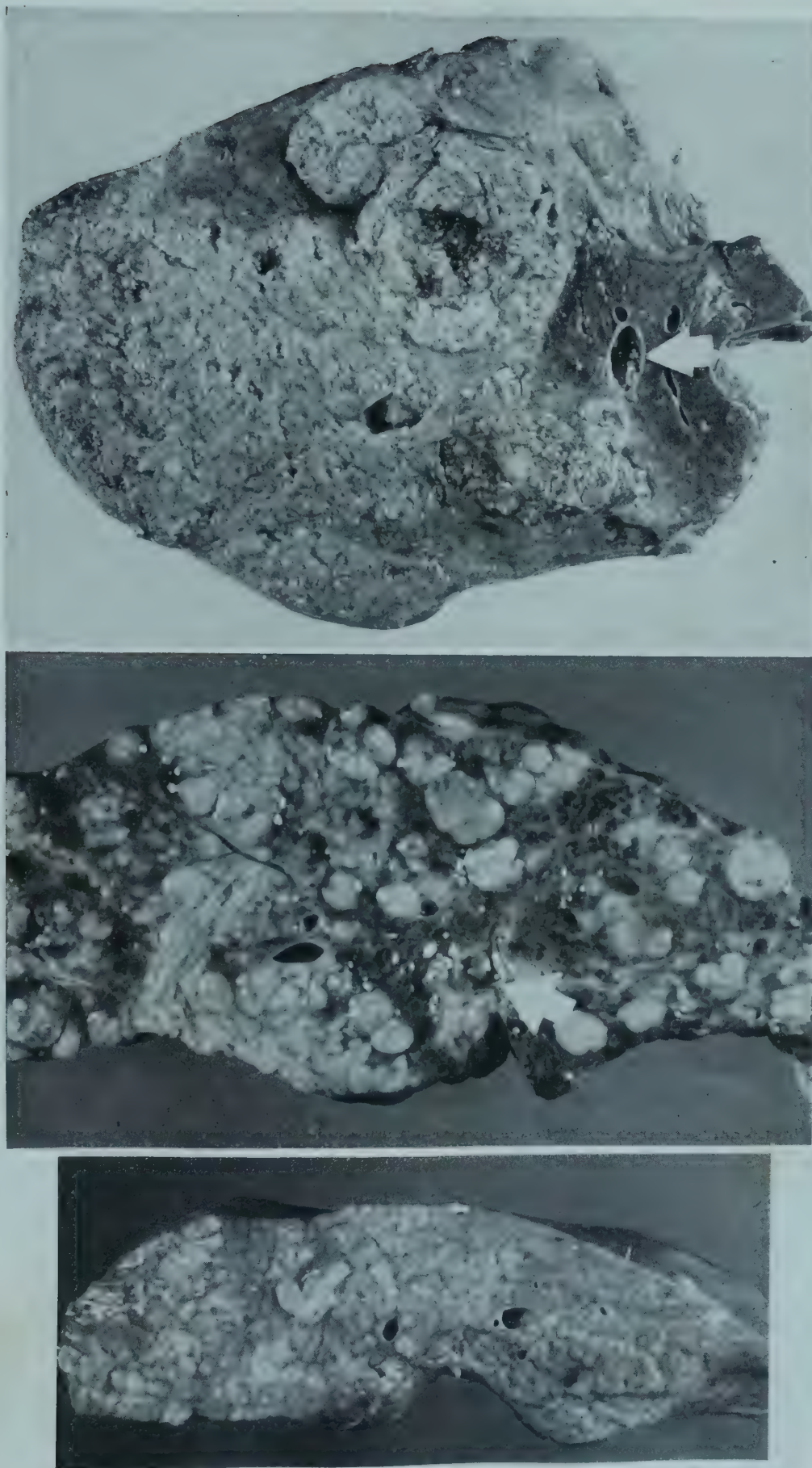


FIG. 195 Primary hepatic carcinoma. *Top.* Massive type. *Center.* Nodular type. (Arrow points to carcinomatous thrombus in portal vein.) *Bottom.* Diffuse type.

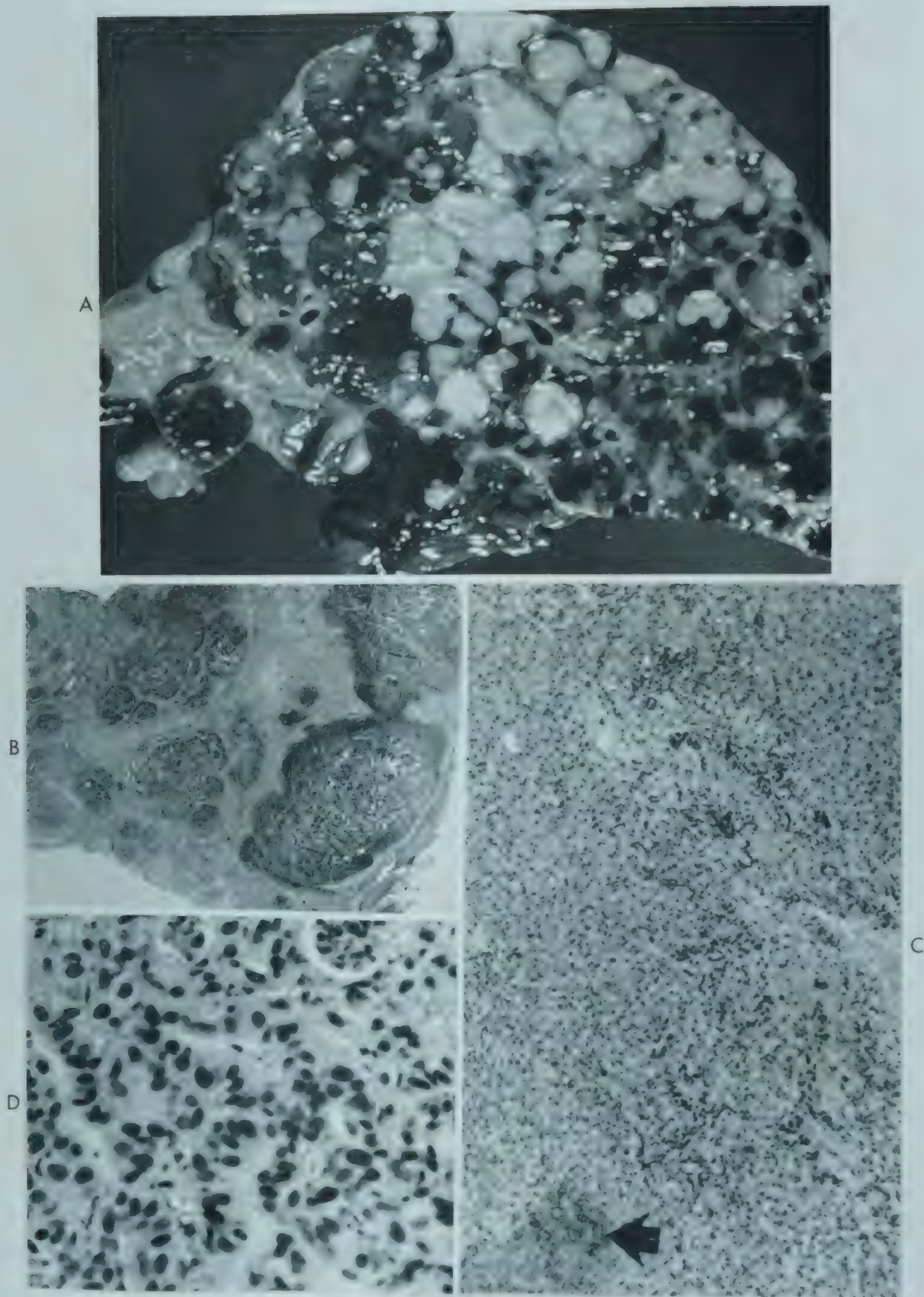


FIG. 106 Primary carcinoma in cirrhotic liver. A Gross picture. B Tumor nodules of various size with central necrosis (Mallory's aniline blue [$\times 3$]). C Biopsy specimen showing necrosis in carcinoma nodule (arrow) (H&E [$\times 60$]). D Detailed view of same carcinoma (H&E [$\times 250$]).

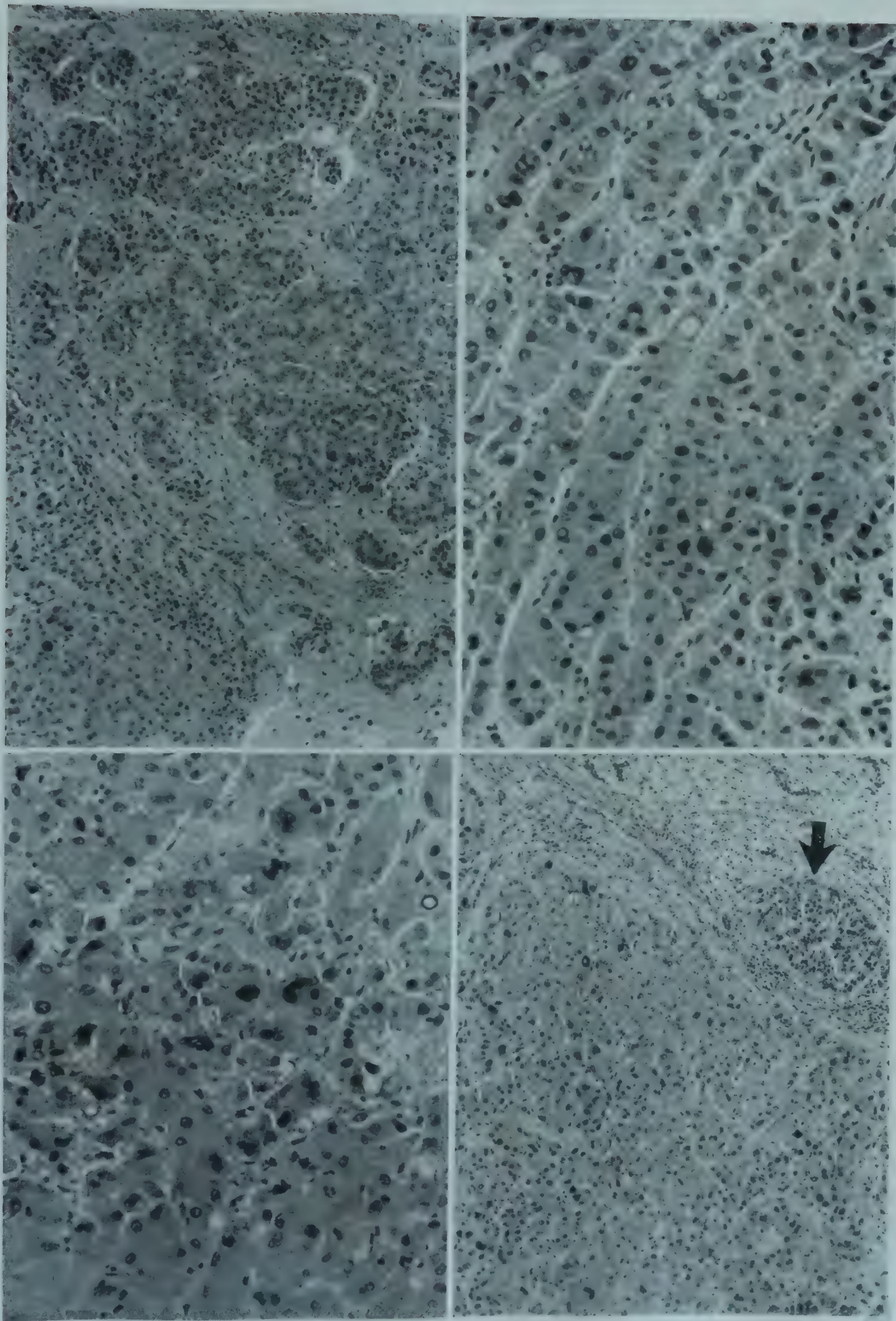


FIG. 197 Primary hepatic carcinoma. H&E. *Upper left.* Biopsy specimen of trabecular type. The trabeculae are usually composed of several layers of cells ($\times 90$). *Upper right.* Trabeculae composed of carcinoma cells varying in shape and staining qualities and arranged as blastemata several cells thick. The cytoplasm appears homogeneous ($\times 230$). *Lower left.* Multinucleated tumor giant cells ($\times 150$). *Lower right.* Tumor growth in portal vein branch (arrow) ($\times 65$).

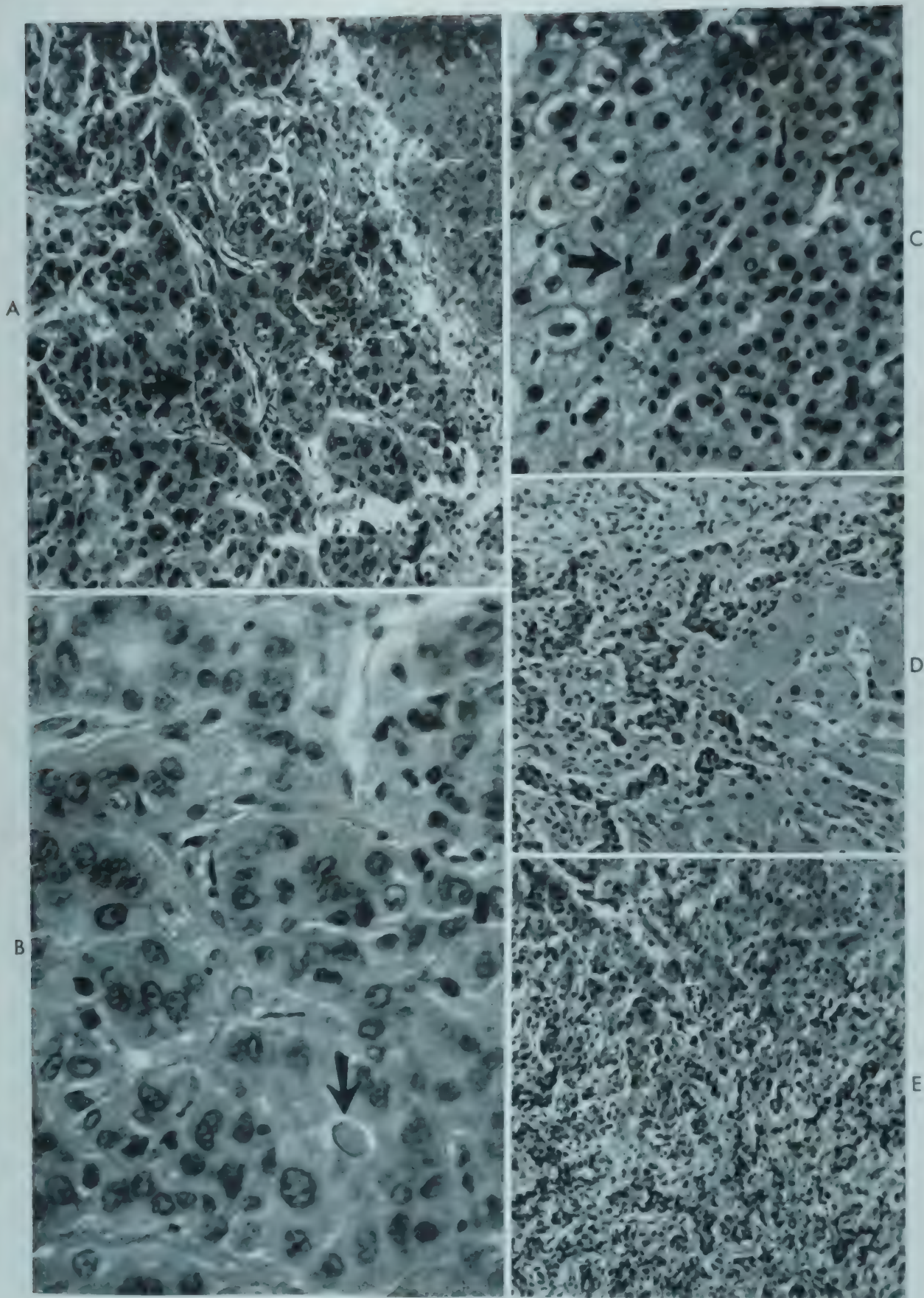


FIG. 198 Primary hepatic carcinoma. H&E. A. Biopsy specimen showing trabeculae of tumor cells arranged in a glandlike fashion, with transitions to ductules (arrow) ($\times 130$). B. Detail of A. Note transition between hepatic and ductular tumor cells and plugs in the lumens of the glandlike structures (arrow) ($\times 315$). C. Biopsy specimen showing border between hepatic and tumor cells, which have some ductular features (arrow) ($\times 240$). D. Connection between carcinomatous hepatic cells and ductlike structures probably derived from compressed hepatic cells ($\times 140$). E. Ductular cell carcinoma invading liver parenchyma ($\times 115$).

1. Development of thin tumor-cell plates simulating normal liver plates.

2. Exaggeration of the trabecular patterns, with formation of thick, fingerlike compact extensions appearing as cords, sometimes containing central bile canaliculi.

3. Formation of thick columns, appearing as sheets in sections, consisting of irregularly arranged, usually undifferentiated epithelial cells with no resemblance to liver structure.

4. Widening of the lumen to a degree that the apparent acini resemble those of the thyroid gland.

5. Fatty metamorphosis of parts of the tumors not parallel with the fat content of the liver itself.

6. Discoloration of the carcinoma by bile pigment granules in the tumor cells and bile plugs in bile canaliculi of the tumor, while the normal liver tissue is not necessarily bile stained. Bile formation is frequently more conspicuous in extrahepatic metastases than in the liver itself. This is often seen also in tumors with a ductular appearance, indicating their hepatocellular nature.

7. Formation of giant cells by enlargement and increase in the number of nuclei and increase of cytoplasm with or without fat vacuoles or bile pigment (Fig. 197, lower left). Predominance of such cells produces the relatively rare giant-cell carcinoma, in which the original trabecular structure is usually absent.

8. Progressive anaplasia, causing the hepatic cells to become spindle-shaped and to resemble sarcoma cells. Some hepatic sarcomas or angiosarcomas reported are probably varieties of primary hepatic carcinoma, especially if such a carcinoma is present in other areas of the same liver (see Carcinosarcoma, later in this chapter).

9. Arrangement of the epithelial cells around vessels, simulating a perithelioma [888, 2090].

10. Ductular and ductlike structures frequently seen in primary hepatic carcinoma result from four processes: (a) The central bile capillary in a hepatic-cell plate widens, with or without simultaneous reduction of the granularity and disappearance of the cytoplasmic basophilia of the lining hepatic cells (Fig. 198B); (b) Actual bile ductules and ducts form from hepatic cells near fibrotic areas. Usually the stroma condenses around the ducts and forms a basement membrane. The epithelium may become ciliated, as in embryonal stages. Occasionally the cells become high columnar. Large nodules composed of ductules develop. The influence of growth stimuli upon the bile duct system is suggested by unexplained dilatations of

the common bile duct [95]; (c) Two-cell-thick hepatic-cell plates appear compressed by surrounding excessive connective tissue and thus resemble ductules in tissue sections [910] (see Bile Ductules, Chap. 15). They remain in contact with the neighboring carcinoma, but the structures themselves are apparently not carcinomatous (Fig. 198D); (d) Irregularly arranged carcinomatous plates invading the surrounding tissue appear similarly compressed by connective tissue and resemble ductules (Fig. 198E). The desmoplastic reaction around hepatocellular plates, trabeculae, sheets, or cords seldom becomes massive, although it may be severe around necrotic areas.

11. Necrosis, especially in the center of the tumor nodules, is the result of interference with the blood supply (Fig. 196B, C). This occurs in the expanding parenchymal nodule and even earlier during intravascular growth. It is enhanced by the disturbance of the blood flow resulting from preexisting cirrhosis. The early changes are karyolysis and the presence of coarse basophilia in the cytoplasm of the shrinking cells. Eventually, after disappearance of nuclear and cytoplasmic staining, a shadowy structure persists. The lining cells of the sinusoids become necrotic later and finally also disappear. Liquefaction may destroy the cancer nodule completely.

12. Hemorrhage, sometimes extensive, usually accompanies necrosis. A special variety, in which hemorrhage is so severe that the possibility of a vascular tumor is considered, is the "hemorrhagic hepatoma of Espérance" [2715]. Hemorrhage is aggravated by the low prothrombin level often caused by cirrhosis and diffuse hepatocellular degeneration.

Grading. A grading of primary hepatic carcinoma based upon the dedifferentiation of the cytologic characteristics and trabecular structure has been attempted [888]. Grade I is a lesion simulating noncancerous hyperplasia if unquestionable carcinoma is present in other areas. Grade II carcinoma strongly resembles liver plates. In grade III, the plate structure is coarsened or abolished and the cells are dedifferentiated, while in grade IV this has proceeded to an anaplastic medullary growth resembling a sarcoma with loss of cohesiveness of the cells. The value of this grading is limited by the fact that all grades occur simultaneously more frequently than in carcinomas of other organs.

Extension of Tumors. Extension of the tumor follows four main patterns.

CENTRIFUGAL GROWTH. Centrifugal nodular growth leads to compression of the surrounding hepatic tissue, which shows pressure atrophy and fibrosis. In this type of nodular growth the tumor is fairly sharply demarcated (Fig. 196*B*). Interference with the blood supply by tumor emboli may produce massive necrosis and collapse, followed by the formation of septums. Regeneration stimulated by loss of liver tissue leads to "collateral hyperplasia" [240]. Consequently, cirrhosis may develop around tumor nodules. In single sections, especially of biopsy specimens, this type of cirrhosis can not be separated from diffuse preexisting cirrhosis. Direct extension of the cancer leads to peritoneal spread and diaphragmatic invasion. This is less common than spread to the regional lymph nodes and to the lungs.

PERISINUSOIDAL EXTENSION. The cancer invades the surrounding parenchyma either through the perisinusoidal tissue space or through the sinusoids. Hepatic-cell plates are replaced by cancer cell plates during perisinusoidal spread. The absence of a sharp dividing line gives the impression of a multicentric transition of normal hepatic cells into cancer cells. Such a replacement can take place in preexisting cirrhotic nodules, which then appear to consist of tumor cells.

VENOUS SPREAD. Primary hepatic carcinoma has a characteristic tendency to invade the branches of the portal vein, which are the draining veins of the carcinoma (Fig. 197, lower right). The tumor grows from the smaller branches in a retrograde fashion into the larger branches, including the main stem, and frequently it follows portal venous channels throughout the liver. Many grossly visible cancer nodules are actually tumor thrombi in dilated veins. In addition, fragments broken from the tumor thrombi produce intrahepatic metastases. Systemic invasion is, in part, prevented by the compression of hepatic vein tributaries owing to the associated cirrhosis. As a rule the hepatic veins are invaded when the portal vein branches are obstructed. Rarely do metastatic tumors in the liver invade grossly visible portal vein branches. Hypernephroid carcinoma may be an exception. But thrombosis of the main stem of the portal vein occurs frequently in carcinomas other than primary hepatic cancer, such as carcinoma of the stomach, pancreas, and biliary tract.

DISTANT METASTASES. Extrahepatic metastases histologically resemble the original cancer and may form bile or contain fat. The arrangement of the

tumor cells is best seen in lymph node metastases. Extrahepatic metastases develop frequently by the venous route, to the pancreas via the portal vein and to the lungs via the hepatic veins. Regional lymph nodes, lungs, and retroperitoneal lymph nodes are the most commonly involved sites, in the order listed [2090]. Bone metastases are found in about 3 per cent of the cases [240] (Fig. 199*B*). The average incidence of metastases at the time of death is about 60 per cent in the United States, and metastases are far more frequent in Africa or the Orient, where carcinoma develops at an earlier age [240, 1562]. Despite extensive venous invasion within the liver, metastases may be absent, indicating a relatively low biologic malignancy of the individual cancer cells [888]. Various statistical surveys list the organs to which metastases occur, and few seem to be exempt [240, 1531, 3253].

Unusual Types of Primary Hepatic Carcinoma.

MIXED HEPATOBILIARY CARCINOMA, OR CHOLANGIO-HEPATOMA. Such a tumor usually represents differentiation of primary hepatic carcinoma into hepatocellular and ductular elements and does not differ from the common type [32, 888, 2816, 3498]. Very rarely, however, two independent tumors are noted in the same liver, one grossly white and hard, consisting of hepatic cancer cells. Metastases, if present, are composed of only one type. This rare lesion seems to be a coincidence of primary hepatic carcinoma with a cancer of the intrahepatic bile ducts (see Carcinoma of the Intrahepatic Bile Ducts, Chap. 59).

CARCINOMA IN HEMOCHROMATOSIS. While the surrounding cirrhotic liver contains much iron pigment, the tumor itself is iron-free, and its color contrasts it sharply from the surrounding tissue. Iron-free ductular cells, compared to the iron-containing noncancerous ductules in this disease, seem to indicate the first step in malignant degeneration [888, 3492].

CARCINOSARCOMA. This rare lesion, combining sarcomatous with carcinomatous features, probably results simply from spindle-shaped anaplasia of the hepatic cells.

Primary Hepatic Carcinoma in Childhood and Infancy. In the first year of life, primary carcinoma of the liver is the most common malignant epithelial tumor, sharing a relatively high incidence with neuroblastoma and renal adenomyosarcoma, or Wilms's tumor [886]. The tumor appearing in the neonatal period, sometimes called "hepatoblastoma," is apparently embryonal in nature and pro-

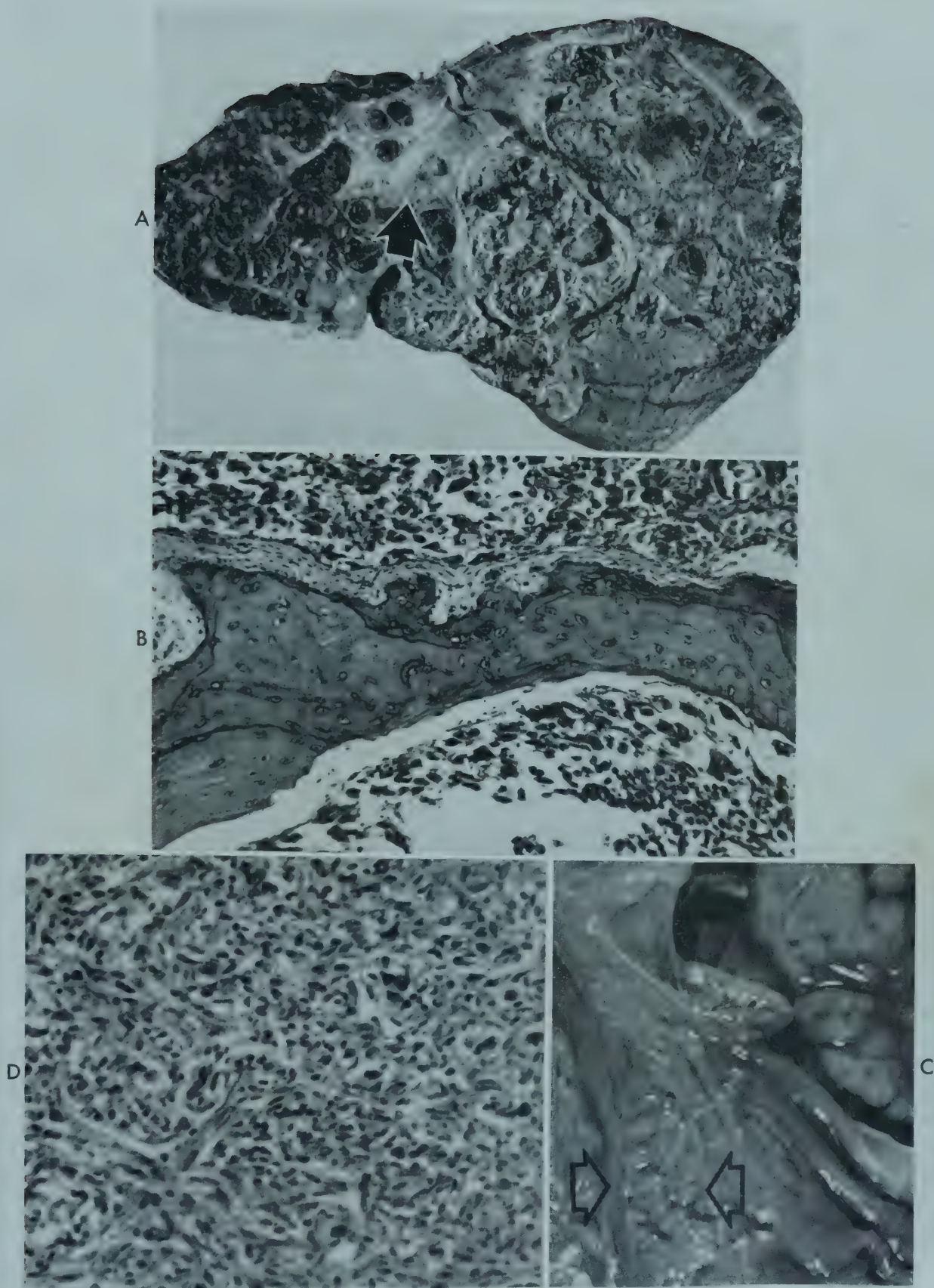


FIG. 199 A. Massive primary hepatic carcinoma of a child one year old. Note the extensive areas of fibrosis (arrow). B. Trabeculae of primary hepatic carcinoma metastasizing to bone marrow. H&E ($\times 115$). C. Carcinomatous thrombus in main stem of portal vein. D. Primary undifferentiated sarcoma of the liver. H&E ($\times 125$).

contain cartilage, osteoid tissue, bone, striated muscle, and even areas of squamous-cell epithelium [886, 3617]. Bizarre histologic features, including cylindromatous changes, are seen, and sometimes the differential diagnosis from neuroblastoma is difficult. Cirrhosis is hardly ever noted in the surrounding parenchyma, but extramedullary hematopoiesis may be abundant. A series of such tumors has been reported [275, 3194, 3348] (Fig. 199A). Clinically they usually present themselves first as right-sided abdominal masses. No tendency for a hereditary factor is apparent [886]. The tumors have to be separated from hamartomas with parenchymal or mesenchymal predominance and from focal hyperplasia or bile duct cysts. They also have to be differentiated from hemangioendotheliomas, rare sarcomas, and "adrenal rest" tumors.

Clinical Differential Diagnosis. Three clinical problems arise in the diagnosis of primary hepatic cancer: (1) unexplained hepatomegaly; (2) differentiation of carcinoma with cirrhosis from uncomplicated cirrhosis; (3) differentiation of primary carcinoma from metastatic carcinoma or other tumors in the liver or neighboring organs.

Clinically fever and pain are important characteristics (see Clinical Manifestations, under Human Primary Hepatic Carcinoma, earlier in this chapter). In the laboratory, increase in the alkaline phosphatase activity and in gamma globulin may be of help. Roentgenologically, elevation of the diaphragm and calcifications in the liver are seen [2911, 3347]. Splenoportography has been used to visualize hepatic nodules. [565, 2840] (see Hepatosplenoportography, Chap. 38). Peritoneoscopy with liver biopsy is a useful diagnostic tool [3621].

Structural Differential Diagnosis. **GROSS FEATURES.** The absence of cirrhosis speaks somewhat against primary hepatic carcinoma, while the presence of tumor nodules in a cirrhotic liver should arouse suspicion of such a tumor, since metastases from other carcinomas in a cirrhotic liver are relatively rare [2030]. Umbilication of nodules is less frequent in primary carcinoma than in metastases. Invasion of the portal veins rarely occurs in cancers other than primary hepatic ones, with the excep-

tion of hypernephroid carcinoma or carcinoma of the tail of the pancreas.

MICROSCOPIC FEATURES. In biopsy specimens, two problems arise: (1) the differentiation of primary carcinoma of the liver from nonneoplastic liver tissue, especially regenerative nodules seen in cirrhosis; (2) the differentiation of primary from secondary carcinoma.

Carcinoma vs. Regenerative Nodule. Anaplasia with an increased nuclear cytoplasmic ratio and giant cells suggests carcinoma. Nevertheless in small biopsy specimens the separation of a well-differentiated carcinoma from a very active regenerating nodule is sometimes difficult. In these instances, invasion of veins may represent the only conclusive evidence of malignancy. Sometimes the carcinoma cells are smaller than the normal hepatic cells and plates are irregularly arranged. A sharp border between normal and carcinomatous tissue facilitates recognition of carcinoma (Fig. 198C).

Primary vs. Secondary Carcinoma. Primary rather than metastatic carcinoma is suggested if the cytoplasm of the tumor cells is granular and eosinophilic, or if bile pigment is present in the cytoplasm or in bile casts. The same is suggested by transitional structures between hepatic-cell plates and ductules, with or without increase in the surrounding connective tissue (Figs. 196D, 198A, B, C). Intravenous growth not adjacent to tumor nodules strongly suggests primary carcinoma, since metastatic carcinomas invade only portal vein branches in contact with tumor nodules. The presence of cirrhosis is not reliable, because perifocal lesions around the tumor may appear cirrhotic in biopsy specimens. Desmoplasia and intrasinusoidal growth speak for metastases. Endothelial cells are found between cancer cells in metastatic tumors, while endothelial cells are found only on the outside of the tumor in primary hepatic carcinomas [888].

Therapy. In recent years carcinoma of the liver has become surgically interesting because of the chance of removal of a tumor if it is detected early [2816]. Such removal is facilitated by better knowledge of the distribution of vessels and bile ducts [424, 1076, 2508, 3498].

CARCINOMA OF THE BILIARY PASSAGES, NONEPITHELIAL TUMORS, AND METASTATIC TUMORS OF THE LIVER

CARCINOMAS OF THE BILIARY PASSAGES

Carcinoma of Intrahepatic Bile Ducts

Bile duct carcinomas were formerly considered more common than hepatocellular carcinomas, but with better understanding of hepatic histology and embryology and with the classification of many cholangiocellular carcinomas as primary hepatic carcinomas, carcinomas derived from interlobular and larger intrahepatic bile ducts have become relatively rare. The clinical and pathologic features of these tumors differ distinctly from those of the primary hepatic carcinoma. Intrahepatic bile duct carcinoma is rather rare in the tropics. No sex difference is found, and the tumor is rarely associated with cirrhosis. Invasion of the portal veins is uncommon. The tumor is always unicentric. The connective tissue stroma participates to a much greater degree, and metastases are more frequent than in the nontropical form of primary hepatic carcinoma [240, 888]. A relation to hepatolithiasis has been assumed [2883]. Carcinoma of the intrahepatic bile ducts biologically behaves as carcinoma of the extrahepatic bile ducts.

Clinical Manifestations. The symptoms are not significantly different from those of primary hepatic carcinoma, and most statistics list them together [888]. Hepatomegaly, abdominal swelling and pain, weight loss, and jaundice are the most frequent complaints. Jaundice seldom becomes as deep as in primary hepatic carcinoma. Bile duct carcinoma arising in the larger bile ducts at the hilus of the liver clinically and pathologically is an intermediate form between carcinoma of the intrahepatic bile ducts and the extrahepatic bile ducts (Fig. 200). Early biliary obstruction with jaundice is the predominant clinical finding, and the liver is enlarged. This is a diagnostically

troublesome site, because the extrahepatic biliary ducts appear narrow and collapsed at operation, and even the surgical differentiation from intrahe-



FIG. 200 Primary carcinoma at the bifurcation of the hepatic duct spreading into the hilar connective tissue (arrows).

patic cholestasis is very difficult. Despite metastases to regional lymph nodes and the lungs, life expectancy in bile duct carcinoma is slightly longer than in primary hepatic carcinoma [888].

Structural Alterations. Grossly, the livers are usually heavier than those with primary hepatic carcinoma, and usually one circumscribed, gray-white, firm nodule is present, with small daughter nodules irregularly distributed throughout the liver. Histologically, a fairly mature adenocarcinoma with glandular and alveolar, ductal, or papillomatous structures is found, as in any other adenocarcinoma [240, 888, 1562, 2816, 3253]. Differentiation of the lesion from metastases from carcinoma of the extrahepatic bile ducts or gallbladder is impossible. Differentiation from other adenocarcinomas, such as those from the gastrointestinal tract, especially the colon, from the pancreas, from a bronchus, or from an ovary, is also virtually impossible. The basic cell is a cuboidal to columnar epithelial cell; the bigger the bile duct from which it originates, the taller it is. Hilar carcinoma, therefore, usually has a high epithelium. The cytoplasm is clear, loose, and faintly acidophilic or granular. Mucus is occasionally produced. The lumen often contains a pink material if no mucus is present. Around the glandlike and papillomatous structures a distinct basement membrane is noted, which may become hyalinized. Severe desmoplasia gives rise to scirrhous formation. Undifferentiated tumors are rare. Vessels are far less frequently invaded than in primary hepatic carcinoma and if invasion is present, the vessels contain papillomatous structures attached to their walls. If cirrhosis is associated with bile duct carcinoma, it is considerably less advanced than in hepatocellular carcinoma.

Carcinoma of the Extrahepatic Bile Ducts

Carcinomas of the extrahepatic bile ducts are relatively common tumors of major clinical and surgical importance. Their detailed description is not within the scope of this book; reference is made to reviews. Carcinoma of the extrahepatic bile ducts is more common than that of the gallbladder, according to recent statistics [1778, 2421]. The differentiation of the carcinoma of the extrahepatic biliary ducts from the carcinoma of the papilla of Vater is not always easy, although the latter originates from the duodenal epithelium. In some instances of far-advanced tumors, the separation of carcinoma of the pancreas from that of the common bile duct is difficult.

Etiology. The role of intraductal gallstones, as well as of preceding inflammation with stricture, has been widely discussed [1188, 1778, 2421]. In all series the incidence of gallstones in the ducts

or the gallbladder is much higher than would be expected in the particular age and sex groups. The figures vary from 30.6 per cent [1778] to 57 per cent [2421]. Inflammatory lesions and cholecystitis are noted in more than 20 per cent of all cases in all series, but the evidence for an etiologic connection is doubtful, since the inflammatory lesion may be secondary to the carcinoma [3617]. An effect of carcinogenic material in bile has been assumed [1056]. Whether papillomatous proliferations precede carcinoma formation is questionable, since the carcinoma itself is seldom papillomatous and is more often infiltrating.

Incidence. The majority of cases occur between fifty and seventy years of age, and slight male predominance is found in all larger series. The carcinoma occurs more commonly in the common duct than in the main hepatic duct, with an apparent predilection for the junction between the hepatic and cystic ducts. Carcinoma of the cystic duct is rare [984].

Clinical Manifestations. Jaundice occurs in about 90 per cent of the cases. It is the first symptom to appear and usually is initially painless. The feces are acholic, and the urine is dark, but at surgery or autopsy a probe can often be passed through the duct, precluding complete mechanical obstruction. This has elicited much discussion as to the mechanism of jaundice [1778, 2421]. Part of the cholestasis is mechanical, in the form of constriction of the wall of the common duct by tumor invasion, plugging of the lumen of the duct by the tumor mass, or constriction by adjacent metastatic lymph nodes. Interference with peristalsis, because of stagnation of the bile flow, and invasion of the nerves [2421] may be additional components not apparent on anatomic examination. The next symptom of importance is pain, which is present in about 50 per cent of cases, possibly from invasion of the nerves. Weight loss, melena, and cachexia are also found in about half the cases before death; they usually appear late. The liver is enlarged in more than half the cases, while the gallbladder is felt in less than half the cases. Severe anemia is found in only a few instances, and chills and fever are rare. Terminally many patients show metastases with ascites and edema, especially if death in cholemia has been postponed by surgical construction of a biliary fistula.

Laboratory Findings. Moderate leukocytosis and acholic feces are present, and the duodenal contents are free of bile or contain very little. Fungus

tionally, the findings of extrahepatic cholestasis, with increasing and often complete obstruction, predominate, and hepatic-cell damage occurs late or in the presence of bacterial infection. Fluctuating cholestasis with a temporary drop of the serum-bilirubin level occurs less frequently than in carcinoma of the papilla of Vater, where it results from sloughing of the tumor.

Structural Alterations. The pathologic findings are those of an infiltrating and constricting adenocarcinoma, which may become very desmoplastic and scirrhous. The early lesion is a plaquelike hardening of the wall, owing to mucus-producing tumor cells in all layers. Papillomatous growth into the lumen is less frequent, and anaplastic medullary carcinoma is rare [1778]. Below the tumor the ducts are collapsed; above it and in the liver they are dilated. When tumors involve the common duct or the cystic duct, the gallbladder is usually greatly dilated, more often than is suspected clinically. The infiltrating and medullary carcinoma more frequently produces metastases and extension into the hepatic hilus and the structures in the hepatoduodenal ligament than does adenocarcinoma. Metastases probably occur later than with carcinomas of the pancreas or gallbladder but are usually found at operation or at autopsy. The most common site of metastasis is the liver, followed by the regional lymph nodes, the pancreas, and peritoneum, while relatively few develop in the lung. Generalized extensive spread is rare [2728]. Hepatic complications include infected biliary hepatitis and secondary biliary cirrhosis. In addition, empyema of the gallbladder, liver abscesses, biliary peritonitis, and atrophy of the pancreas may occur.

Therapy. Some tumors near the ampulla can be radically excised, while in others, palliative bypassing of the obstruction by internal or external fistulas is the only recourse to avoid death in cholemia [1188]. Many surgeons pessimistically feel that life expectancy is scarcely prolonged by these procedures. In general, the average period of survival after operation is only a few months, and after the onset of jaundice, not more than half a year [2421].

Carcinoma of the Gallbladder

Carcinoma of the gallbladder is of major clinical interest, both because it is possibly a sequela of cholelithiasis and cholecystitis, and because of the chance for a surgical cure [97, 1188, 1778, 2325, 3477, 3617].

Etiology. In 75 to 100 per cent of the cases of carcinoma of the gallbladder, gallstones are present [97, 1188, 1778, 2325, 3196]. This raises the question whether calculi cause carcinoma or result from it. Insertion of foreign bodies and carcinogenic material into the gallbladder produces a proliferative mucosal reaction resembling carcinoma, but most authors question its malignant character [97, 1778, 2325]. Some seem to have succeeded in producing metastasizing lesions [2580]. The evidence for the causative role of calculi of the gallbladder in man is based on (1) the development of carcinomas in patients carrying gallstones for many years; (2) the absence of gallstones in metastatic carcinoma of the gallbladder; (3) the presence of carcinogenic agents in bile and the known chemical relations of cholesterol and bile acids to carcinogens [1056, 3196]. Stones may develop secondary to the carcinomatous ulcer, as they do in cholecystitis, and stones have been found in metastases within the liver from carcinoma of the gallbladder [3683]. The causative role of gallstones in carcinoma is therefore not established, and some investigators doubt that such a relationship exists [1778], although most authors are inclined to accept it [1188, 3196]. This raises the question as to whether the possibility of malignant degeneration is an indication for cholecystectomy. Carcinoma is found in 1 to 15 per cent of patients with gallstones [2325], probably the most correct figure being around 3 per cent [3196]. About 5 per cent of patients with gallstones reaching the age of sixty-nine develop cancer of the gallbladder [1188].

Cholecystitis plausibly is a causative agent of carcinoma, especially if inflammation leads to extensive proliferation. However, the statistical evidence is not convincing, especially since inflammation is usually associated with carcinoma.

Incidence. Carcinoma of the gallbladder is far less common than carcinoma of the gastrointestinal tract. It occurs in approximately 4.5 per cent of all carcinomas and in less than 0.5 per cent of all necropsies [97]. At the time of gallbladder operation for any cause it has been found in slightly more than 1 per cent of cases. The age incidence is similar to that of carcinomas in general, the peak being in the sixth and seventh decades. A female preponderance of approximately 3:1 is in keeping with the sex incidence of cholelithiasis and in contrast to that of carcinoma of the liver or the biliary tract [97, 1778]. Its incidence in Negroes is not higher than in white persons. In Mexican women

and in Japanese and Filipinos it seems to be more common and not necessarily associated with a higher incidence of biliary calculi [3196].

Clinical Manifestations. The differential diagnosis from cholelithiasis and cholecystitis with which the tumor is so often associated is difficult [97, 1188, 1778]. Pain and jaundice are present in about two-thirds of the cases; hepatomegaly, a palpable mass, and localized tenderness in about half the cases; and edema and ascites in approximately one-third of the cases. Preceding symptoms referable to the gallbladder, such as colic and dyspepsia, are found in about half the cases, and in about 6 per cent surgery had been performed on the gallbladder [97].

Anemia does not seem to be a constant finding, and other laboratory findings are of little help. Roentgenologic studies usually do not assist except in determining whether there is failure of visualization of the gallbladder.

Structural Alterations. If the associated inflammation is cicatrizing, the gallbladder is small. If the tumor is in the neck, the gallbladder is greatly enlarged because of obstruction of the cystic duct, and symptoms occur early. In desmoplastic lesions the wall is very thick.

The majority of the tumors are adenocarcinomas. They are (1) papillomatous, with mainly endophytic growths, producing a cauliflower-like lesion, which consists of anaplastic epithelium thrown into villi, while the wall is only moderately infiltrated, and extensive growth over the mucosa is rare [3245]; (2) infiltrating and usually anaplastic, producing grossly visible diffuse thickening of all layers of the wall, with desmoplasia progressing to scirrhous formation; (3) colloid-producing. Almost all adenocarcinomas produce some mucus, but in either the papillomatous or the infiltrating type this characteristic sometimes dominates the picture and numerous signet ring cells are found.

Statistically, infiltrating carcinoma seems to be the most common, and colloid carcinoma the least common [97], although mixtures occur frequently. Squamous-cell carcinomas account for about 4 per cent of the total [97]. They have elicited much discussion [2580], since squamous metaplasia does not occur in the noncarcinomatous gallbladder [3617]. The papillomatous type seems to have a slightly better prognosis [97].

METASTASES. Carcinoma of the gallbladder spreads early into the surrounding tissue, especially into the liver and the bile ducts. It may also

extend into any of the surrounding structures, and perforation is fairly common, so that an internal gallbladder fistula should arouse suspicion of carcinoma. Biliary obstruction leads to biliary hepatitis, which may become infected and progress to hepatic abscess formation. Metastases develop early and are found in almost all cases at autopsy [1778]. Hilar and other regional lymph nodes are often involved. Systemic metastases often cause symptoms, while the primary site remains small and undetected [97, 2804]. Despite extensive surgery, including wide excision of the adjacent parts of the liver, the prognosis is poor and 5-year survivals are unusual [97, 1188]. Small carcinomas found incidentally at surgery and removed radically are exceptions.

NONEPITHELIAL TUMORS OF THE LIVER AND BILIARY TRACT

The majority of vascular tumors of the liver are hamartomas (see Hepatic Hamartomas, Chap. 57). A small group of highly malignant hemangioendothelial or undifferentiated sarcomas (Fig. 199D) is derived from the Kupffer cells, and differentiation of these tumors from anaplastic carcinoma is often difficult [1413]. In rare instances they result from the internal irradiation effects of Thorotrast injections [2155]. Other tumors derived from the mesenchymal elements are extremely rare [2804, 3498]. These include lymphangiomas, lipomas [3683], fibromas [3498], fibrosarcomas [3018], rhabdomyosarcomas, leiomyosarcomas, and neurogenic tumors. "Adrenal rest" tumors and primary melanoblastomas of the liver are anatomic curiosities. Teratoid tumors also are seldom seen [2304]. In the biliary tract, fibromas, lipomas, neuromas, and melanomas, as well as carcinoid tumors, have been reported [583], and in the gallbladder, fibromas [3039] and sarcomas have been found.

METASTATIC TUMORS IN THE LIVER

Next to the regional lymph nodes the liver is the most frequent site of metastasis of any tumor, and metastatic carcinoma of the liver is far more frequent than primary carcinoma [10], except in cirrhosis.

Types of Tumors. Carcinomas of almost all organs metastasize to the liver. The liver contains metastases in approximately 30 per cent of all cases in which carcinoma is found at autopsy and

in half of the cases in which the carcinoma is in the splanchnic system [2804, 3012, 3617]. The liver is the site of predilection for metastases from carcinomas of the pancreas, colon, rectum, stomach, lower part of the esophagus, and gallbladder. Almost the same percentage is found in carcinoma of the breast, while the incidence is lower in carcinoma of the lung, kidney, and ovary, and in all these tumors the liver is not the site of predilection. The liver is relatively rarely involved in carcinoma of the uterus and of the oral cavity. Melanoblastomas and neuroblastomas frequently metastasize to the liver and produce tremendous enlargement, sometimes with the primary lesion being obscure. If carcinoma of the liver is found in men, stomach, lung, and colon are the probable sites, while in women breast, colon, stomach, and uterus should be suspected, in the order listed. Sarcomas metastasize to the liver less frequently than carcinomas. The liver is frequently involved in leukemia, lymphoma, and myeloma (see *Abnormal Hematologic Structures in the Liver*, Chap. 60).

Routes of Invasion. Spread to the liver occurs by direct extension by the venous route and by lymphatic spread. Direct spread occurs from carcinomas of the gallbladder, mainly into the right lobe, from carcinomas of the bile ducts, and, less frequently, from carcinomas of the stomach, pancreas, and colon. Venous invasion usually follows along the portal vein, and retrograde embolism through the hepatic veins from the heart is rare. Portal vein distribution favors the location of carcinomatous metastases from the stomach, pancreas, or ascending colon in the right lobe and from the sigmoid or rectum in the left lobe, according to the streamlines of flow in the portal vein system (see *Streamlines of Flow*, under *Portal Vein*, Chap. 18). However, this distribution is not found regularly and is often scattered. Lymphatic spread to the liver is therefore common from organs in the portal system. The lymphatic route is the preferential route from breast and lung tumors, probably via mediastinal lymph nodes. Within the liver, spread occurs either by the venous route [3616] or, less frequently, through the lymphatic vessels, which appear distended by tumor nodules in the portal tracts (Fig. 201A). Direct extension from the periphery of tumor nodules occurs within the sinusoids and infrequently in the perisinusoidal spaces [888]. The tumor nodules differ from the surrounding liver by receiving mainly arterial blood [388, 2745]. As

the tumor becomes larger the portal blood supply entirely disappears. The blood drainage apparently is mainly through the portal vein branches similar to structures in the portal tracts [3660], carcinoma metastases thus distort the arterial tree in the liver and alter hepatic circulation.

Clinical Features. Frequently carcinoma metastases in the liver produce no clinical manifestations. If they do, they are hepatic enlargement with palpable nodularity, hepatic pain and abdominal distention with local discomfort, fever, malaise, and anemia. As the process progresses, cachexia and rapid hepatic enlargement with ascites and jaundice become apparent.

CLINICAL SIGNIFICANCE. Metastatic carcinoma in the liver presents three clinical problems: (1) when metastatic carcinoma in the liver is found or suspected and the primary site is not known; (2) when a known carcinoma is suspected of having spread to the liver, important from the point of view of surgical therapy and prognosis; (3) when widespread malignancy is present and the patient follows a downhill course, the rapidity of which depends on the degree of malignancy of the primary tumor and not upon the hepatic involvement. The number of cases falling into each of these three groups is probably equal. In a very small fourth group, carcinoma with hepatic metastases produces no clinical manifestations, but the cancer is found incidentally at operation or autopsy.

HEPATOMEGALY AS A PRESENTING FEATURE. Metastatic hepatic carcinoma presents the problem of diffuse hepatomegaly or nodular enlargement with or without jaundice. If large firm nodules are palpated the problem is simple. The enlargement of the liver is not influenced by bed rest, dietary or cardiac management. As a rule a slight fever with leukocytosis is present. When ascites, edema, anorexia, and weight loss develop, the diagnosis often becomes obvious, while laboratory findings and liver biopsy are the most important guides until this time. Splenic enlargement occurs only if ascites is present as the result of portal vein obstruction and therefore is a late symptom.

JAUNDICE. Jaundice is a frequent symptom only in terminal stages [3502]. If it is present, it is caused by one of three factors:

1. The extrahepatic biliary ducts are obstructed by the primary tumor (in the extrahepatic biliary ducts or head of the pancreas), by extensions of the primary tumor (in the gallbladder or stomach), or by metastases to the connective tissue at

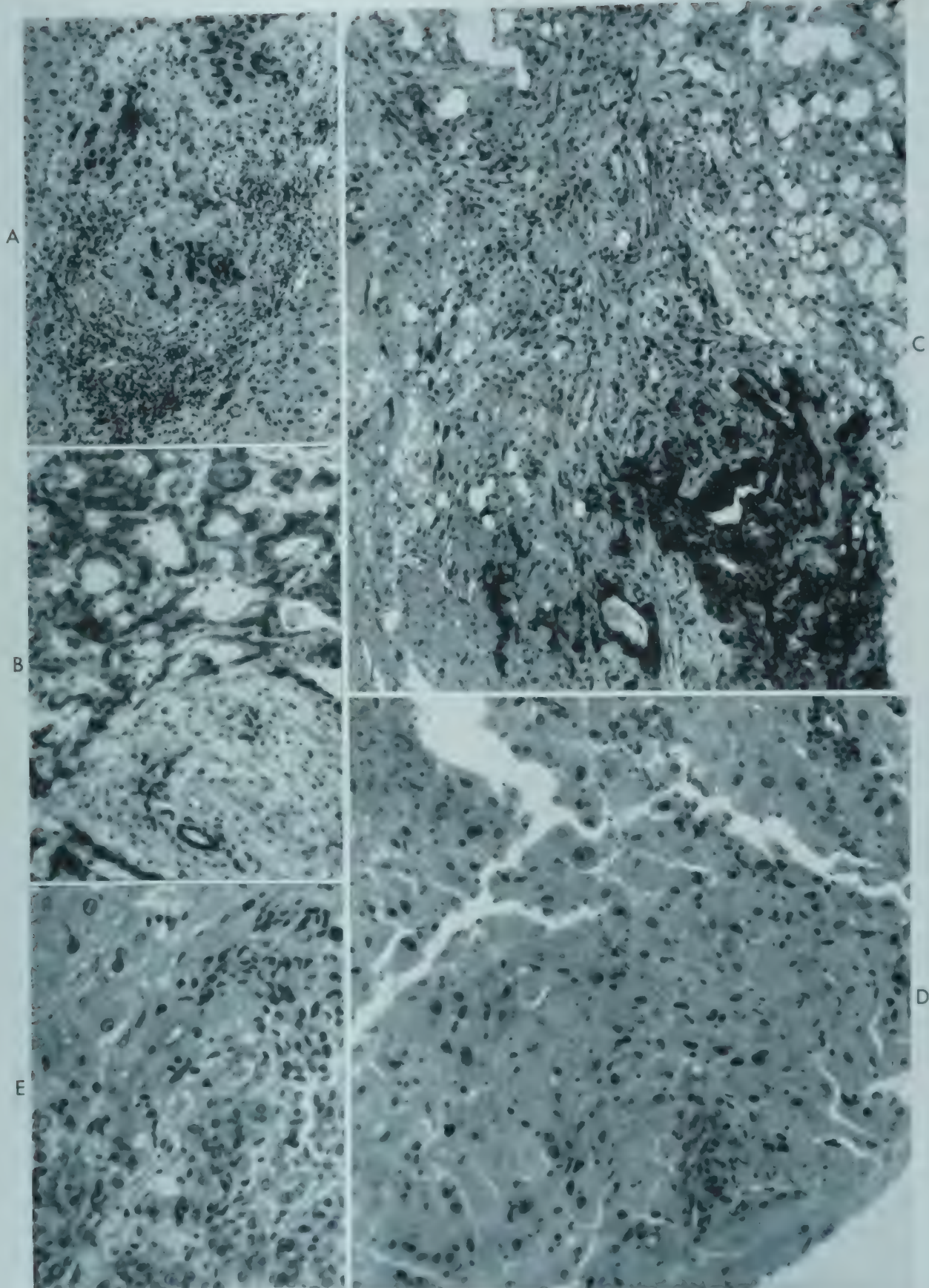


FIG. 201 Biopsy specimens of secondary carcinoma of the liver. H&E. A. Spread of bronchogenic carcinoma in the lymphatic vessels of a portal tract ($\times 125$). B. Mucus-producing adenocarcinoma of the colon replacing the hepatic parenchyma. The portal tracts are preserved ($\times 270$). C. Adenocarcinoma of the pancreas metastasizing into a liver with fatty cirrhosis ($\times 95$). D. Hypernephroid carcinoma. Note similarity of the tumor cells to hepatic cells or, even more, to cells of primary hepatic carcinoma ($\times 170$). E. Metastatic melanoblastoma in liver ($\times 195$).

the hepatic hilus, the ducts, or the periportal lymph nodes. The adventitial tissue of the ducts is invaded, fixed, and compressed. Obstructive jaundice produced by metastatic carcinoma has been said, in view of scarcity of reports in the recent literature, to be rare [523, 1465], but in large autopsy series such cases are found rather frequently.

2. Intrahepatic obstruction or angulation of the bile ducts by the expanding nodules has been considered an important feature [2267], and focal bile stasis is noted histologically near metastases. However, the correlation between jaundice and the number and size of carcinoma metastases is poor [3077].

3. Hepatocellular damage in the form of non-specific reactive hepatitis is an important feature in the production of jaundice, although the relation between functional and structural changes is rather confusing [2867].

DETECTION OF METASTASES IN PROVED CARCINOMA. When detection of hepatic metastases in a proved carcinoma is the problem, palpation of the liver, radiologic examinations with the demonstration of a high diaphragm or of nodes within the liver, hepatic tests, and liver biopsy are useful tools.

Laboratory Findings. The results of almost all hepatic tests are abnormal in more than 20 per cent of cases. Carcinoma, especially of the gastrointestinal tract, produces abnormal results even without metastasis [7]. Cephalin-flocculation, thymol-turbidity, and hippuric acid-synthesis tests are of little help in the detection of metastases; the only tests to which some value can be attached are those of Bromsulphalein retention [152, 2267, 2543, 3031, 3322] and alkaline phosphatase activity [2267, 3031, 3077]. Increased Bromsulphalein retention, caused mainly by disturbed circulation, is out of proportion to the results of the flocculation tests and arouses suspicion of hepatic metastasis in the absence of jaundice [3031]. Increased serum-alkaline phosphatase activity was found in 90 per cent of patients with advanced metastases and in 44 per cent of patients without hepatomegaly [2267], although in an autopsy series small carcinoma metastases were not associated with increased serum-alkaline phosphatase activity [3077]. Bone metastases should be excluded as the cause of the increased alkaline phosphatase activity. Hepatomegaly with normal serum-gamma globulin level and no jaundice in the presence of high mucoproteins speaks for metastatic carcinoma

and against cirrhosis, granulomatous hepatomegaly, and primary carcinoma. Alkaline phosphatase increase and Bromsulphalein retention in the absence of jaundice, hepatic enlargement, or nodularity speak for carcinoma metastases if bone involvement and cardiac failure are excluded. Normal results of these tests do not exclude carcinoma metastases. The serum-gamma globulin level is usually normal and becomes elevated only in the instances of extensive metastases. Evidence of extrahepatic cholestasis of short duration with associated hepatocellular damage suggests the possibility of malignant obstruction of the extrahepatic bile ducts with metastases in the liver [3077].

Structural Alterations. Carcinoma metastases to the liver vary from multiple small nodules to large ones of different colors, depending upon the type of tumors, e.g., melanoblastoma metastases may be black. Necrosis in the center of nodules accounts for umbilication in those which protrude from the surface. Obstruction of portal flow around nodules leads to Zahn infarcts in their vicinity. Invasion of portal veins and hepatic arteries produces anemic infarcts, in which the carcinomatous origin is hardly discernible. Extensive hemorrhage can occur into the nodules; for instance, in choriocarcinoma metastases. Sometimes the metastases are very small and diffusely infiltrating, grossly resembling small nodular cirrhosis. Exceptionally diffuse infiltration of the tissue spaces enlarges the lobule, producing coarsening of the architecture without visible nodules. Various complications result, such as perihepatitis with extensive adhesions and carcinomatous peritonitis, intraperitoneal hemorrhage, secondary infection with subsequent supuration, and portal vein invasion and thrombosis. Hepatic vein obstruction causes diffuse enlargement of the liver. Metastases in a cirrhotic liver are rare [2030], with the exceptions of secondary biliary cirrhosis produced by a malignant obstruction [3471] and of early fatty cirrhosis (Fig. 201C). Probably the disturbance of the circulation interferes with the seeding of metastases. Cirrhosis does not seem to influence the development of carcinomas anywhere else in the body [1353].

Newer Diagnostic Methods. Tools useful in the detection of tumor metastases, in addition to the hepatic tests, include splenoportography (see Hepatosplenoportography, Chap. 38), or survey of hepatic radioactivity after administration of radioiodinated human serum albumin [3685]. Tumor

cells can be found in ascitic fluid. Peritoneoscopy and liver biopsy have also been applied [602, 1162, 1172, 1795, 2867]. By biopsy, various types of carcinoma can be recognized (Fig. 201). Metastases from hypernephroid carcinomas may resemble cirrhotic hepatic parenchyma (Fig. 201D). In the largest series reported, the incidence of positive biopsy findings was surprisingly high (77 per cent) whether the liver was nodular or not [2867]. On the other hand, cases appearing to have metastatic carcinoma clinically were proved by biopsy to have other diseases. The incidence of positive findings increases if the specimen is inspected grossly and if liver biopsy is repeatedly performed. Although many cases go undetected because of the focal nature of the lesion,

liver biopsy by now is probably the most efficient method of establishing the presence of hepatic metastases.

Therapy. In recent years, with improved knowledge of surgical anatomy of the liver and with greater experience in partial hepatectomy, the surgical removal of metastases of the liver has become an accepted procedure [2321]. This procedure appears feasible if only one or two metastases are present, and it is palliative and sometimes even curative if the primary lesion is also removed. Irradiation of the liver has also been recommended for palliation but has little lasting effect. In some tumors, such as dysgerminoma or neuroblastoma, the metastases regress after surgical removal of the primary lesion.

PART VII

*Internal and External
Environment and the Liver*

RELATION OF LIVER TO SPLEEN AND HEMATOPOIETIC SYSTEM

HEPATOLIENAL RELATIONSHIPS

From 15 to 40 per cent of the portal blood reaching the liver comes from the spleen. Therefore, changes in the circulation in one organ profoundly influence the circulation of the other. The spleen plays an important role in the metabolism of blood pigment and thereby influences the bile pigment metabolism in the liver. Since the spleen and liver together contain the bulk of the reticulo-endothelial system of the body, some stimuli produce parallel changes in both organs. A description of the histologic structure of the spleen as the basis for the understanding of these relations has been presented under portal hypertension (see Portal Hypertension, Chap. 29).

Influence of the Liver upon the Spleen. The liver influences the spleen by causing either portal hypertension or reactive splenitis from irritation by hepatic breakdown products. Since the reticulo-endothelial elements are capable of tremendous proliferation, the spleen may be very large in liver diseases, particularly if cellular and hydromechanical factors are combined [1227, 3186].

Influence of the Spleen upon the Liver. Few facts are known about the influence of the spleen upon the liver. Splenectomy is said to lead to the appearance of lymphatic foci in the portal tracts of the liver, which take over some of the functions of the spleen. Furthermore, increased hemolysis as the result of hypersplenism produces all the effects discussed in Chapter 49 (see Hepatocellular Degeneration from Hemolysis, Chap. 49). Most instances of hypersplenism are not induced by hepatic disorders but are either the result of a primary splenic disorder or are secondary to such diseases as lymphosarcoma, Hodgkin's disease, tuberculosis, and sarcoidosis.

Parallel Effect upon the Spleen and Liver. Some of the splenic changes which occur in liver disease can not be explained as a consequence of hepatic disorders but are concomitant alterations, apparently produced by the same injurious substances which damage the liver, even though the nature of these substances is rarely established. Sometimes the splenic changes are far more conspicuous than the hepatic alterations. The name "Banti's syndrome" has been applied to the manifestations of hypersplenism caused by portal hypertension (see Banti's Syndrome, under Sequelae of Hypertension, Chap. 29). The underlying disease is portal or splenic vein thrombosis or cirrhosis. Occasionally conspicuous enlargement is associated with hypoplastic anemia, rather than with hemolysis, while the portal vein is free and the liver shows only minimal cirrhosis. In such instances of "splenomegalic" cirrhosis, parallel injury to the liver and spleen has to be assumed.

In some forms of acute hepatitis in man and in experimental animals, changes in the spleen, such as rupture of the reticulum framework primarily in the pulp cords, point to simultaneous damage. The irregular arrangement of the pulp cords makes the demonstration of this rather difficult. Such lesions stimulate new formation of fibers, so that the splenic enlargement with fibrosis may possibly be explained not only on a hydromechanical basis but also as the result of simultaneous injury.

Since hypersplenism is a possible consequence of hepatic disorders, the question arises when it should be the indication for splenectomy. Theoretically, this indication is present in almost every patient with an enlarged spleen, since removal of the spleen would reduce the blood flow to the esophageal veins. In addition, collaterals develop

in the adhesions around the splenectomy site, producing communications between the short gastric and the gastroduodenal veins on one side and the veins of the peritoneum draining to the vena cava on the other. However, in practice, splenectomy is rarely, if ever, beneficial in any of the hepatic disorders, except in the few instances where the hemolytic component dominates the entire picture. Furthermore, unless a splenorenal shunt is made at the time of splenectomy the effect on portal hypertension is fleeting.

RELATION BETWEEN LIVER AND HEMATOPOIETIC SYSTEM

Hepatic Hematopoiesis

The liver is the site of hematopoietic activity in the embryonal period and under abnormal circumstances in postnatal life, either as a compensation for depressed bone marrow function or as part of a systemic reaction. Therefore the participation of the liver in many hematologic conditions does not represent an influence of the hematopoietic system upon the liver.

Fetal Hematopoiesis. In fetuses more than 13 mm long, hematopoietic foci are found in the hepatic anlage and persist until birth [1659]. Portal and intralobular hematopoietic foci give the embryonal liver a characteristic appearance [3259]. In immature fetuses they are very extensive [2608]. In the mature fetus the portal tracts contain a collar of hematopoietic tissue, and foci are found within the lobular parenchyma (Fig. 60, lower right). Whether they derive from extravascular mesenchymal elements or from capillary endothelium within the blood spaces, or from both locations, is unsettled [114]. In diseases, especially anemia and syphilis, these foci are more prominent than normally. In hemolytic disease of the newborn, their tumorlike appearance resulted in the name "erythroblastosis fetalis."

The hematopoietic foci disappear during the first months of postnatal life, but some small lymph follicles in the portal tracts persist throughout the adult life (see Embryology, Chap. 20).

Postnatal Hematopoiesis. **BONE MARROW REPLACEMENT.** Bone marrow depression in postnatal and adult life, resulting from invasion of the bone marrow by carcinoma [1659], by Hodgkin's disease [3630], by osteosclerosis, or by leukemia [1659] and from various anemias, including per-

nicious anemia, causes hematopoietic foci to appear in the liver in the lobular parenchyma and occasionally in the portal tracts. Cells of the erythrocytic and myelocytic series and occasionally even megakaryocytes appear.

INTOXICATIONS. Experimental saponin intoxication of rabbits produces hematopoietic foci in the liver. In man, radium, benzol [2190], and a variety of other drugs [3672] which alter the bone marrow cause extramedullary hematopoiesis, although this is usually less intense in the liver than in the spleen. In the liver, the foci within sinusoids sometimes form small nodules, which dilate the sinusoids and simulate granulomas. This is usually associated with proliferation of Kupffer cells containing iron pigment.

IDIOPATHIC MYELOID METAPLASIA. Hematopoiesis occurs in the liver in myeloid metaplasia [307] or panmyelosis [295]. In this condition the bone marrow alterations are considered part of a generalized mesenchymal reaction [2546], in which the liver participates [295, 2546]. The liver is usually much enlarged and shows the hematopoietic foci with the predominant changes in the parenchyma [295, 2546] (Fig. 202, lower left). Many megakaryocytes may be found in the sinusoids. Diffuse pleomorphic proliferation of the medullary and extramedullary reticulum is seen, with differentiation into all blood elements. In the portal tracts sometimes the megakaryocytes are the most prominent feature and may even be the only one. Areas of focal necrosis with subsequent fibrosis are often found. Some investigators feel that the liver is responsible for the lesion by failing to detoxify various poisons [3672]. In some cases the liver possibly only filters out cells formed elsewhere [2546]. Some authors consider panmyelosis an independent entity [295, 307, 2546], while others consider it a form of aleukemic myelocytic leukemia [1449]. The hepatic changes in myeloid metaplasia, chronic myelocytic leukemia, and even erythroleukemia are best considered as manifestations of allied myeloproliferative disorders [712].

Abnormal Hematologic Structures in the Liver

Leukemias and Lymphomas. **MYELOCYTIC LEUKEMIA.** The liver is enlarged and gray-red in color and the lobular markings are not easily discernible [295, 1053, 1303, 2324]. Histologically, the portal tracts are extensively infiltrated by myeloid elements in the leukemic stage but less infiltrated in aleukemic or subleukemic stages. The sinusoids

and the tissue spaces contain many leukemic cells. The Kupffer cells give peroxidase reactions similar to those given by myelocytic cells [2469]. The relative involvement of parenchyma and portal tracts varies [295], and sometimes the portal tracts alone are infiltrated. Typical myelopoietic foci are often found in leukemia in liver biopsy specimens [3040]. The histologic appearance of the liver does not necessarily aid in the differentiation from lymphatic leukemia or in the diagnosis of leukemia, since the liver sometimes is spared entirely and is also normal in the preleukemic phase [308]. Moreover, liver biopsy is dangerous in leukemia.

MONOCYTIC LEUKEMIA. The origin of monocytic cells from the Kupffer cells can be observed in some instances, but portal infiltration is the most frequent finding.

LYMPHOCYTIC LEUKEMIA. In lymphocytic leukemia, the lobular pattern of the liver is either exaggerated or obscured by irregular enlargement of the portal tracts, which sometimes appear as grossly visible nodules [1303]. Histologically, infiltration of the portal tracts by lymphatic elements is conspicuous. They surround vessels and bile ducts without compressing them.

LYMPHOSARCOMA. Large white tumor nodules of varying size are noted, with a homogeneous cut surface except where they are necrotic. They resemble metastatic carcinoma grossly. Histologically, in both the round-cell and reticulum-cell types of lymphosarcoma, the nodules in the portal tracts are present and the surrounding parenchyma is sometimes infiltrated through the tissue spaces, as demonstrated in liver biopsy specimens [1795, 3440]. In larger nodes, the relation to the portal tracts is no longer apparent. The appearance in round-cell lymphosarcoma resembles that of lymphocytic leukemia (Fig. 202, upper left), while reticulum-cell sarcoma resembles anaplastic carcinoma (Fig. 202, upper right). The liver may be involved in giant follicular lymphoma.

HODGKIN'S DISEASE. The liver is involved in approximately half the cases of sarcomatous or granulomatous Hodgkin's disease [3285, 3474]. Grossly, tumor nodules of varying size, degree of necrosis, hemorrhage, and fibrosis are sometimes seen (Fig. 203, upper). They resemble lymphosarcoma and usually differ from metastatic carcinoma by their polymorphism. The portal lymph nodes may be involved even if the liver is spared grossly. The hepatic lesions are usually small, and

granulomas are found in the parenchyma and portal tracts. In the parenchyma the earliest changes are small proliferations of the Kupffer cells or mesenchymal cells in the portal tracts differentiating into proliferated reticulum cells and Reed-Sternberg giant cells (Fig. 203, lower left and right). The granulomas coalesce and show central necrosis and fibrosis, producing the polymorphism typical of Hodgkin's disease. This is increased by the varying degree of participation of neutrophilic and eosinophilic leukocytes, lymphocytes, and plasma cells. Invasion of veins leads to endophlebitis. The bile ducts are usually preserved. Exceptionally the liver is the only organ involved [3285], and even the spleen may be free. The involvement of the portal tracts may become widespread and produce septal cirrhosis in rare instances [3195]. Hepatomegaly rarely is found in early Hodgkin's disease but is common in late stages. Jaundice occurs in less than 10 per cent of cases [1208] and results mainly from toxic hepatitis produced by tissue-breakdown products and only exceptionally from extrahepatic obstruction by the enlarged portal lymph nodes, which are commonly involved. In exceptional cases, rapidly spreading Hodgkin's granuloma in the liver may produce fatal hepatocellular degeneration, as well as severe intrahepatic cholestasis, by destroying ducts and ductules. Intercurrent hepatitis in Hodgkin's disease is supposed to arrest temporarily the progress of the lymphoma [1547].

Reticuloendotheliosis. In systemic nonlipid reticuloendotheliosis, or Letterer-Siwe disease, which is probably related to Hand-Schüller-Christian disease (see Cholesterol-storage Disease, under Lipid-storage Diseases, Chap. 53) and eosinophilic granuloma or histiocytosis X, portal fibrosis and infiltration by the abnormal cells occasionally occur. This is sometimes associated with jaundice, bilirubinuria, and evidence of hepatocellular damage [568].

Myeloma. In multiple myeloma, hepatic involvement is common, with diffuse or nodular infiltration of the organ by plasma cells (Fig. 202, lower left). This is associated with enlargement of the liver in two-thirds of the cases. Amyloidosis associated with myeloma often involves the arteries of the liver selectively. Abnormal results of the flocculation tests (cephalin flocculation being more abnormal than thymol turbidity) are caused by the globulin changes characteristic of the disease. In about 20 per cent of all cases, results of other hepatic tests are also abnormal [3114].

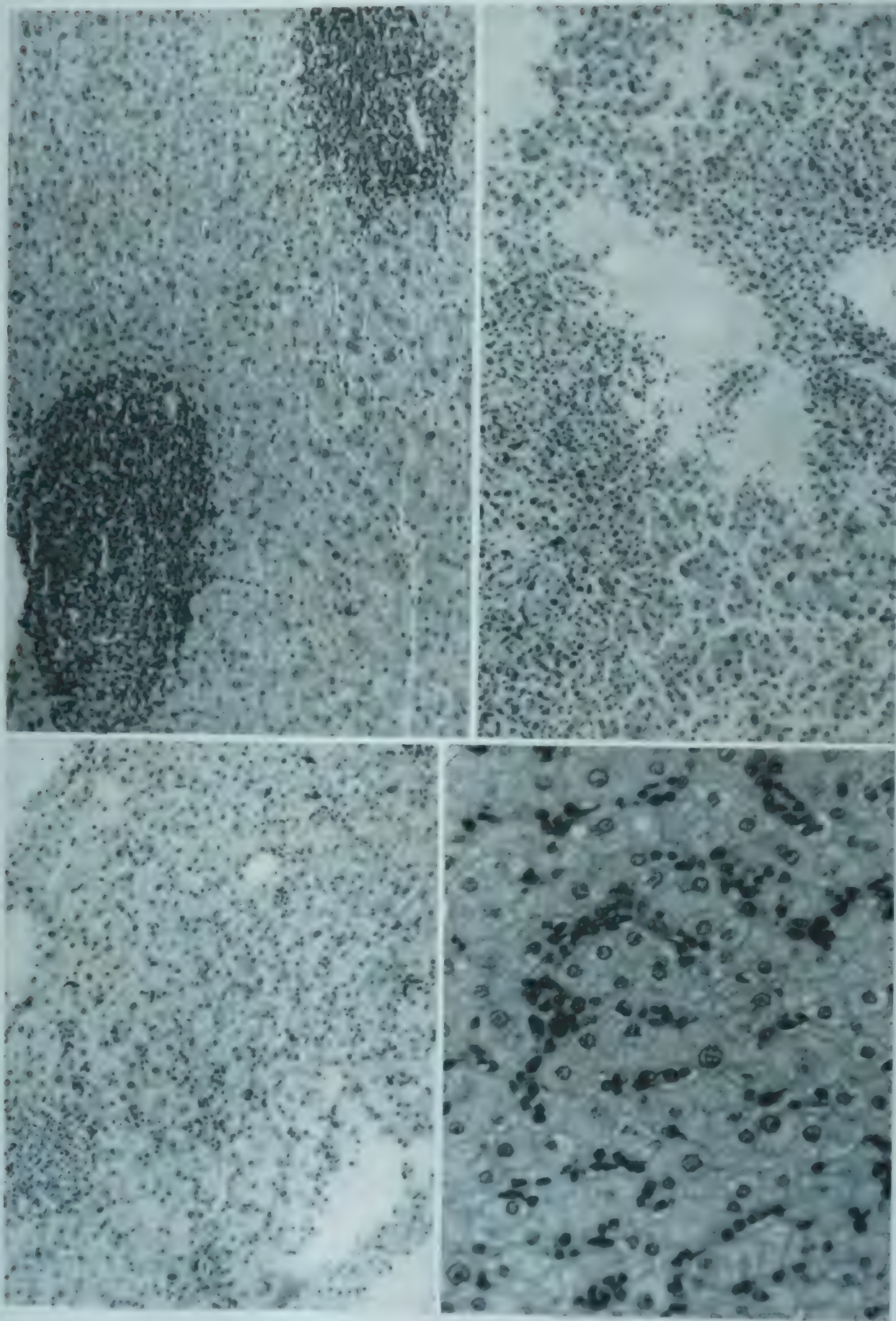


FIG. 202 Liver biopsy specimens—H&E. *Upper left*, Infiltration of portal tracts by round-cell lymphosarcoma ($\times 130$). *Upper right*, Histiocytoma-cell sarcoma spreading from portal tract into the surrounding parenchyma ($\times 110$). *Lower left*, Myeloid metaplasia. Note cell accumulations in the portal tract and lobular parenchyma ($\times 140$). *Lower right*, Multiple myeloma. The sinusoids are crowded with mononuclear cells resembling Kupfer cells ($\times 275$).

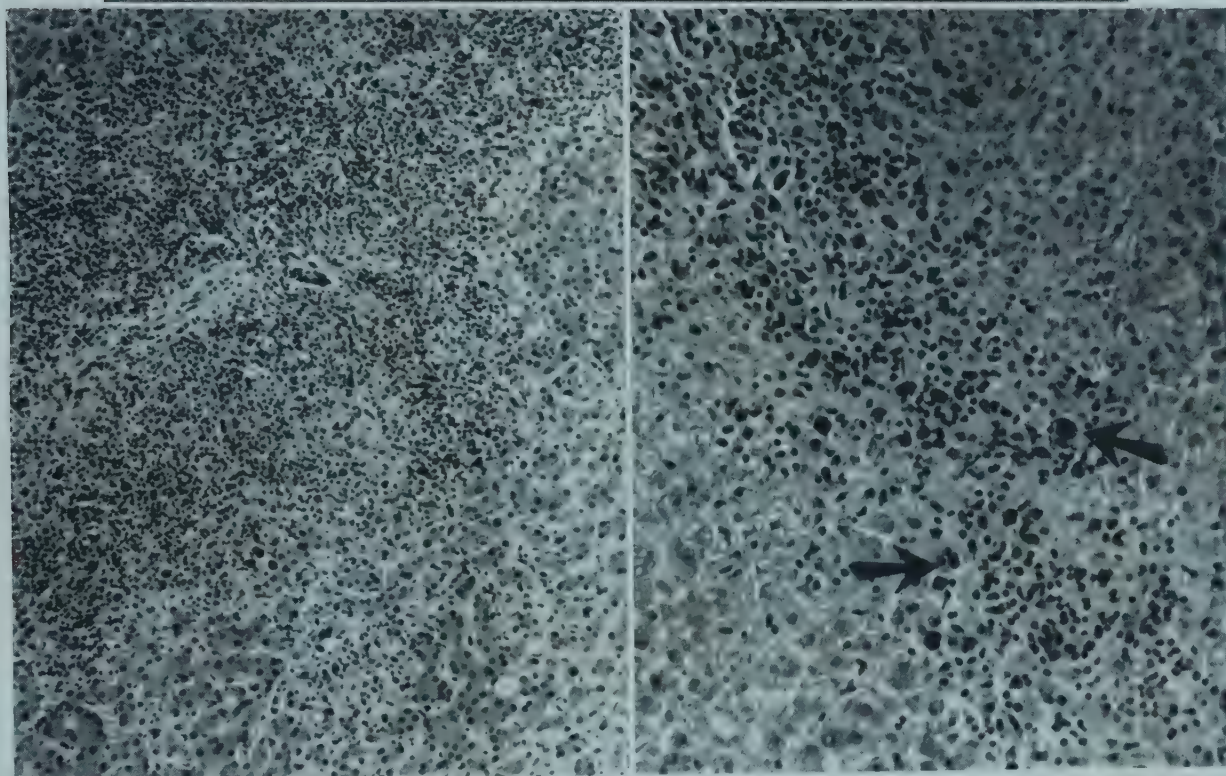
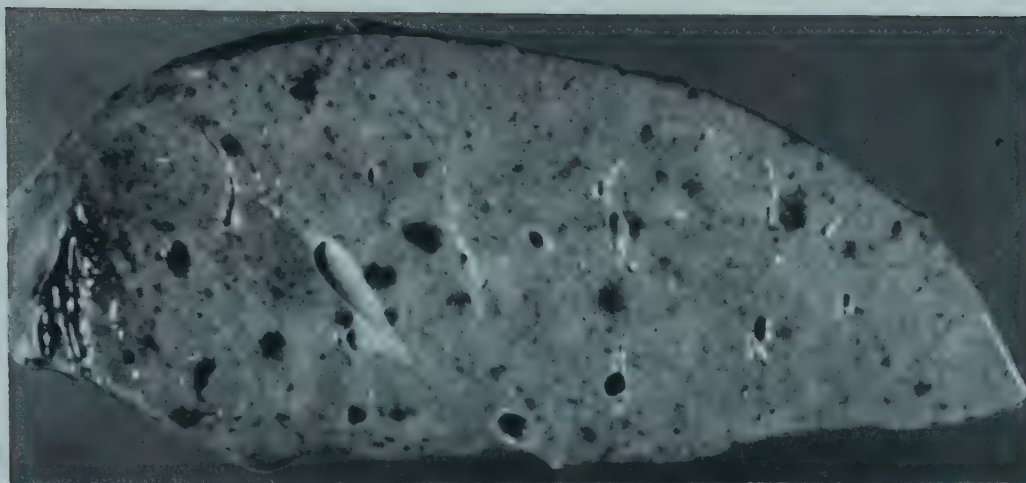


FIG. 203 *Upper.* Portal Hodgkin's granuloma with extensive necrosis, some of which is hemorrhagic. *Lower left.* Polymorphous granulation tissue spreading from the portal tract into the lobular parenchyma, destroying bile ducts and interfering with hepatic circulation ($\times 90$). (Same case as above.) *Lower right.* Detail of same, showing Reed-Sternberg cells (arrows) ($\times 150$).

EFFECT OF THE LIVER ON THE HEMATOPOIETIC SYSTEM

Influence upon Red Cells. NUCLEAR MATURATION FACTOR. The active principle of the erythrocyte nuclear maturation factors is stored in the liver. Production of this principle is faulty in megaloblastic anemias, of which pernicious anemia and sprue are the main examples. Two growth factors, vitamin B₁₂ and folic acid, are parts of an extrinsic factor which forms the maturation factor in the presence of an intrinsic factor in gastric secretion. Although the biochemical defect in pernicious ane-

mia is unknown, lack of the intrinsic factor is probably the cause of the disease. Some nutritional anemias result from lack of extrinsic factor. The macrocytic anemia of liver disease was originally thought to be caused by depletion of the maturation factor because of the inability of the liver to store it [3631]. Assays for the maturation factor in acutely or chronically damaged livers have not conclusively demonstrated any depletion, nor can impaired release of this factor be demonstrated [2273]. Furthermore, administration of liver extract or folic acid fails to influence the usual type of anemia in liver disease [1635].

Megaloblastic maturation arrest has been reported only in a few patients with liver disease and an associated nutritional extrinsic factor deficiency [1635], as well as in a few other instances in cirrhosis [2365] and hemochromatosis [1846]. Therapy with vitamin B₁₂ or liver extract in such instances causes the marrow to revert to normoblastic [1635, 2365].

In addition to storing the maturation factor, the liver probably produces substances which directly influence red cell formation [656] and hemoglobin production [3573]. These substances are reduced in hypoproteinemia.

ANEMIA IN EXPERIMENTAL LIVER DAMAGE. Hepatectomy does not produce anemia in mammals, because they do not live long enough [2202], but it does produce severe anemia in amphibians, which may live 2 to 4 weeks [656]. Heterotopic transplants of liver tissue prevent this anemia. Toxic experimental injury is associated with anemia, which is well correlated with the extent and duration of the lesion.

ANEMIA IN HEPATIC DISEASES. In acute viral hepatitis, macrocytic anemia [1633, 2011] may be masked by hemoconcentration. Anemia is usually more apparent in chronic liver diseases, such as cirrhosis [245, 991, 1633]. It may be explained by nonspecific toxic effects upon the bone marrow, possibly by hepatic-cell breakdown products. Hypersplenism, which increases hemolysis, with development of reticulocytosis and spherocytosis [1633, 3502], or possibly inhibits the release of red cells from bone marrow is more important [245, 991, 2011]. The shortened life span of the erythrocyte in cirrhosis [547, 1633] is in keeping with increased hemolysis. In some instances, the anemia is caused by hypervolemia and the total red cell mass remains normal [178, 1584]. Bleeding, which is common in liver diseases, tends to produce hypochromasia [245].

QUALITATIVE CHANGES IN RED CELLS. Macrocytosis of the circulating red cells is common in hepatic injury, developing soon after its onset [2011]. The red cells appear targetlike (leptocytosis) [691]. Since enlargement compensates for the reduced hemoglobin concentration, and flattening for the increased diameter, the mean corpuscular volume remains normal [2909]. These changes have been explained as being the result of altered formation of erythrocytes [330]. A physical influence upon the circulating red cells seems more likely, however [2011, 2273]. The life span of the red cells is shortened, and therefore the altered appearance

may be a reflection of low-grade hemolysis. Since the degree of jaundice does not influence leptocytosis [330], it is not caused by bilirubin, as has been thought [606], but possibly by bile acids [2273]. Physical factors explain the reduced tendency of the macrocytic flattened red cells to swell, and therefore their fragility is decreased in acute hepatitis as well as in chronic liver damage [330, 606, 2368]. Poikilocytosis is unusual in liver diseases, in contrast to pernicious anemia [2909]. The qualitative changes of the red cells do not necessarily parallel the severity of the disease in man as determined by liver biopsy [245, 1567, 2909]. The presence of macrocytic normochromic anemia may be helpful in suggesting the presence of liver damage but is useless in differentiating the various forms of liver disease.

ALTERATION OF BONE MARROW. Hematopoietic marrow may extend throughout the entire shaft of the long bones, especially in chronic liver disease [943, 991, 3502], and usually shows normoblastic hyperplasia [245, 1005] with a decrease or even reversal of the myeloid/erythroid ratio in human cirrhosis [245, 606] and in experimental animals. Basophilic normoblasts sometimes predominate, but true megaloblasts are rarely found [245, 1005, 2006]. More commonly, giant leukocytes, possibly a reflection of a slight maturation factor deficiency, are seen. Hypocellularity of the bone marrow is seldom found even in advanced liver damage.

Effect on the Thrombocytes. Low thrombocyte counts in hepatic disorders have been repeatedly reported, more often in parenchymal liver disease than in extrahepatic obstruction, and have been considered responsible for some of the bleeding tendency [245, 2350, 3591]. The number of megakaryocytes in the bone marrow is usually slightly increased. The thrombocytopenia is often associated with increased capillary fragility and possibly with vitamin C deficiency. It parallels the elevation of the serum-gamma globulin level and is probably the result of hypersplenism rather than of a direct effect of hepatic injury on megakaryocytes or thrombocytes.

Effect on Segmented Leukocytes. Neutropenia is common in acute as well as in chronic hepatic injury [991, 1005, 2273]. The response to leukocytic stimuli such as infections is dampened. The severity of the disease is not reflected in the degree of leukopenia, and in the marrow neutrophils and eosinophils are increased in cirrhosis. Hypoplasia has been described and is sometimes found in experimental animals [245]. In acute and chronic

hepatitis a shift to the right of the neutrophils with polylobation of the nuclei which does not parallel the degree of jaundice has been reported [3343] and seems to be rare.

Effect on Lymphoid Elements. LYMPHOCYTES AND PLASMA CELLS. In acute viral hepatitis, lymphocytosis occurs in the peripheral blood. Some of the lymphocytes are abnormal. Their nuclei are large, while the cytoplasm is basophilic and contains azure granules. They resemble the atypical cells seen in infectious mononucleosis but are actually toxic lymphocytes. Lymphoid elements are numerous in the bone marrow. Plasmacytosis is not found in acute hepatitis [1944]. In cirrhosis, leukopenia is associated with relative lymphocytosis. Reticulum cells, eosinophils, and especially plasma cells are increased in the bone marrow parallel with the elevation of the serum-gamma globulin level [288, 1635, 2273]. The plasmacytosis may be of such a degree that it raises the suspicion of tuberculosis or multiple myeloma.

LYMPH NODES. The portal lymph nodes are enlarged in most liver diseases and are largest in viral hepatitis, particularly in the chronic stages. Other lymph nodes are also commonly involved in hepatitis. Histologically, nonspecific lymphoid and reticulum cell hyperplasia is seen.

Influence of Hematologic Disorders upon the Liver

Reference has been made to the hepatic changes in hemolytic anemia (see Hepatocellular Degeneration from Hemolysis, Chap. 49) and in leukemias, lymphomas, and allied conditions (see Abnormal Hematologic Structures in the Liver, earlier in this chapter). In severe and protracted primary anemias, hemosiderosis and even hemochromatosis are observed (see Siderosis, or Hemosiderosis, and Hemochromatosis, Chap. 53). In untreated pernicious anemia, little functional impairment of the liver can be demonstrated [2927], while hepatomegaly, liver tenderness, and increased prompt-reacting bilirubin occur in acute anemic episodes [1455, 2217]. In polycythemia vera, jaundice has been reported following hepatic vein thrombosis and after the therapeutic use of chemicals which cause hemolysis.

Parallel Involvement of Liver and Hematopoietic System

Many drugs produce liver injury accompanied by either aplastic anemia (benzol derivatives), hemolytic anemia (phenylhydrazine), or agranulocytosis (sulfonamides) (see Chap. 41).

RELATION OF THE LIVER TO THE GASTROINTESTINAL TRACT AND PANCREAS

RELATION BETWEEN THE LIVER AND THE GASTROINTESTINAL TRACT

The relation between the liver and the gastrointestinal system is influenced by several facts: (1) bile is an important digestive secretion; (2) the liver stores or produces substances which have a specific effect on the gastrointestinal tract; (3) blood from the gastrointestinal tract flows first to the liver; (4) disturbed intestinal absorption deprives the liver of essential nutrients.

Influence of the Liver on the Gastrointestinal Tract

ANOREXIA. One of the earliest symptoms of liver injury is anorexia. This occurs in acute hepatitis and in cirrhosis and may lead to a vicious circle, inducing further liver damage by causing nutritional deficiency [732].

DEFECTIVE ABSORPTION. Defects in digestion and absorption have been postulated in liver disease caused by ascites, altered vitamin B metabolism, reduced intestinal enzymes as a result of protein deficiency, and hypomotility. Reduced excretion or absence of bile because of biliary obstruction or parenchymal liver disease with decreased bile acids in the intestine decreases absorption of fat-soluble vitamins A, D, E, and K, and calcium. It also reduces the alkalinity of the intestinal contents.

Defects in the absorption of protein have not been demonstrated, and fecal nitrogen is not increased even in advanced cirrhosis. Furthermore, in studies of abdominal collateral vein blood, absorption of carbohydrates, fats, and protein was found to be normal [278, 3045]. Also no significant differences were observed between the results of oral or intravenous glucose-tolerance tests [475]. The findings are difficult to interpret because of

possible alterations in intermediary metabolism and because absorbed material may bypass the liver via collaterals [732].

ALTERATION OF GASTRIC ACID AND INTESTINAL MOTILITY. Hypoacidity and atrophic gastritis are frequently seen in liver damage, but the association requires further elucidation [2979], particularly since peptic ulcer and cirrhosis coexist more frequently than would be expected by chance [2023] and since peptic ulcers are found in experimental hepatic injury. Reduced gastric motility with prolonged gastric emptying and decreased intestinal motility have been demonstrated roentgenologically in animals intoxicated with carbon tetrachloride [2743]. Intestinal motility is also reduced in intrahepatic cholestasis [2202]. These changes are not reproduced by ligation of the common bile duct, abdominal vagotomy, or hexamethonium [2743].

INTESTINAL EDEMA. Edema of the intestinal mucosa, often noted in hepatic disorders, is at least partly caused by hypoproteinemia and may be associated with phlegmonous infiltration of the intestinal wall [2613].

PORTAL HYPERTENSION. Portal hypertension leads to the formation of submucous varices in the esophagus, the cardiac portion of the stomach, and the hemorrhoidal veins, all of which tend to bleed (see Portal Hypertension, Chap. 29). It also aggravates edema and hemorrhagic tendencies along the entire gastrointestinal tract [1617]. Massive gastrointestinal hemorrhage may originate from a peptic ulcer or from gastritis even in the presence of esophageal varices [2023, 3264].

Influence of the Intestinal Tract upon the Liver

Excessive or deficient absorption from the gastrointestinal tract influences the liver. Since blood from the superior mesenteric vein flows to the

right half of the liver because of the streamlines of flow in the portal vein, any effect from the small intestine or right half of the colon is more apparent on that side [2202]. Increased absorption of normal and abnormal substances from the intestinal tract possibly is responsible for hepatic injury ("autointoxication").

Whether the liver damage which commonly accompanies inflammatory and ulcerative conditions in the gastrointestinal tract is caused by auto-intoxication, bacterial infection, or faulty absorption is unsettled. Inflammatory changes in the portal tracts are common in any disease of the intestinal tract, and fibrosis of the portal tract with residual lymphocytic infiltration may remain as a scar. Faulty absorption does not explain the functional and structural hepatic alterations seen in peptic ulcer of the stomach or duodenum and in ulcerative colitis, both lesions being in parts of the intestinal tract which absorb nutrients only to a very limited degree. The role of therapeutic diets for these conditions also must be considered.

Peptic Ulcer. The hepatic changes in peptic ulcer seen in both biopsy [2244] and necropsy [1203, 1230] are described under nonspecific reactive hepatitis (see Nonspecific Reactive Hepatitis, Chap. 41). Functional changes in uncomplicated ulcer are unusual. In experimental animals [2742] and in complicated cases they are common [1203, 2354] and include abnormalities in serum proteins [2692], hippuric acid synthesis [2615], galactose tolerance [2270], and citric acid tolerance [3089].

Gastroduodenal lesions, particularly ulcers or diverticula, sometimes kink or compress the common bile duct and cause extrahepatic biliary obstruction.

Ulcerative Colitis. Hepatic involvement was recognized early as a complication of chronic ulcerative colitis, but only a few isolated instances were reported until the last decade [152, 924, 2509]. Recent studies based on clinical [2833], laboratory [2618, 3374], biopsy [1520, 1798, 3374], and necropsy [1655, 1753, 2618, 3493] observations have resulted in disagreement as to the incidence and significance of hepatic changes in this disease. In some large series the incidence of structural alterations in the liver was less than 1 per cent [152, 2765, 2833], whereas in others it was over 50 per cent [1655, 2618]. One possible explanation for discrepancy in the findings is the apparently easy reversibility of most of the hepatic changes [1655].

STRUCTURAL ALTERATIONS. Comparative studies of the necropsy findings in ulcerative colitis and in unselected cases of other diseases failed to reveal an increased incidence of hepatic-cell degeneration [152, 2833] or cirrhosis [1655], but fatty infiltration and inflammatory changes in the portal tracts were found more often in ulcerative colitis than in control series [1753]. Fatty metamorphosis similar to the nutritional fatty liver has been reported in over half of the cases [1655, 2618, 2833, 3493]. Hepatic-cell degeneration and necrosis are much less common [1655, 1753, 1798, 3493] and are related to the severity of the disease. Periductular or portal tract inflammation [152, 1655, 1798] and bile casts in the bile duct with proliferation and fibrosis [1753] are observed, and development of cholangiolitic cirrhosis [152, 1798] has been seen. Whether septal or postnecrotic cirrhosis occurs more frequently in ulcerative colitis than in the general population is unsettled. Less than 50 cases have been reported thus far among thousands of patients with ulcerative colitis [152, 1520, 1655, 1753, 1798, 2618, 3374, 3493]. Most of these cases showed a fine nodular septal cirrhosis often associated with fatty metamorphosis, but several instances of coarsely nodular, postnecrotic cirrhosis have been described [1520, 1798]. Other hepatic changes, such as diffuse inflammation [1798] and multiple abscesses [924, 3358], are rare. The liver is the most common site of metastases from carcinoma arising in the diseased colon [1520, 1655, 1798], and the use of liver biopsy has been recommended for recognition of this complication of ulcerative colitis [1798].

CLINICAL AND LABORATORY FINDINGS. Jaundice [871, 1520, 1655, 1753], hepatosplenomegaly [1520, 1655, 1753], bleeding esophageal varices [1520, 1655], ascites [871], and anemia [2833] have been found in patients with hepatic involvement as a complication of ulcerative colitis. A low serum-albumin level or a reversed A/G ratio out of proportion to the degree of malnutrition has been considered the best indication of hepatic insufficiency in this disease [2618, 3374]. Moderate degrees of Bromsulphalein retention are often present, especially when fatty metamorphosis is found in the liver [1655, 1798, 2618]. Increased prothrombin time is also common [2509, 2618]. The results of the flocculation tests are usually normal [2833], except when cirrhosis is present [1798, 2618].

PATHOGENESIS. Several factors have been held responsible for hepatic changes in ulcerative co-

litis. The one most universally agreed upon is the effect of malnutrition [152, 871, 1655, 1753, 2618, 3374]. This has been related to hypoproteinemia [1655], although no correlation was found between weight loss and fatty liver or toxemia [2618]. The relentless progression of cirrhosis, after colitis has been controlled and nutrition is adequate, has been used as an argument against this etiology [1520]. Toxic factors absorbed from the diseased colon have been accused of producing hepatic changes [1655, 1753, 2618, 2833, 3374]. Such toxic factors also include bacteria brought to the liver via the portal vein [152, 1753, 1798], and toxins resulting from generalized sepsis [152]. Anoxia from anemia has been considered an important factor [152, 2833]. Preexisting or concurrent liver or gallbladder disease accounts for some of the instances [2833], and the use of intestinal antibiotics possibly explains some of the fatty livers [152, 1798]. Recently the possibility that most of the changes, including cirrhosis, result from chronic viral hepatitis or serum hepatitis has been suggested [1520, 1798], but the protracted course has been used as an argument against this theory [152].

Enteritis. Faulty absorption is probably the cause of the fatty liver that rapidly occurs if the small intestine is diseased, as in sprue, regional or tuberculous enteritis, and even tuberculous peritonitis. Diarrhea in infants is usually associated with fatty liver and with focal necrosis. Sometimes jaundice is present. Simple distention of the intestine adversely affects the liver, since it appreciably diminishes portal blood flow [2202].

Gastrointestinal Carcinoma. Carcinomas of the gastrointestinal tract may involve the liver through associated malnutrition, interference with absorption, autointoxication with bacterial as well as tumor tissue-breakdown products, bacterial invasion, and obstruction of the biliary system in addition to metastases. Results of many of the hepatic tests are abnormal even in the absence of metastases [2543].

Effect on the Gallbladder. Embryologically, the gallbladder is an extension of the gastrointestinal tract, and the innervation of the biliary tree is parallel to that of the intestine. Furthermore, the duodenal "hormone" secretin is an important regulator of the flow of bile, apparently both as a choleric and as a cholagogue [1301, 3295]. Therefore, neuromotor disorders of the intestinal tract of various types are associated with biliary dyskinesia. In animal experiments, dilatation of the

colon produces sphincter of Oddi spasm (see Sphincter of Oddi, Chap. 16).

RELATION BETWEEN THE LIVER AND EXOCRINE PANCREAS

The function and structure of liver and pancreas are intimately related because of (1) their close proximity; (2) the drainage of pancreatic blood into the portal vein; (3) the connection between the pancreatic and the biliary ducts.

Bile Duct and Pancreatic Duct Relationship. The pancreas, phylogenetically, has several ducts, only two of which persist in man. The main duct of Wirsung enters the duodenum on the papilla of Vater, together with the common bile duct, and only a small but variable portion of the organ is drained by the accessory duct of Santorini. The duct of Wirsung and the common bile duct enter the duodenum at the papilla of Vater (1) separately; (2) in close proximity so that an impacted stone in either compresses the other or spasm of the sphincter of Oddi constricts both; or (3) with a common terminal channel, permitting reflux in either direction (see Termination of the Common Duct, Chap. 15). Reflux of pancreatic juice into the bile duct is more likely, since the pancreatic secretory pressure is greater than the biliary pressure [824]. The significance of reflux in the pathogenesis of pancreatic disease has been questioned, and acute pancreatitis is often seen in the absence of a common channel [2625, 2720], although biliary tract disease is found in 40 per cent of patients with acute pancreatitis [2545]. Reflux of pancreatic juice into the biliary system has been associated with cholecystitis [3643], but stasis is necessary in addition to reflux; neither alone produces changes [844].

Influence of the Liver on the Pancreas

The Pancreas in Liver Disease. Pancreatic changes occur in chronic liver disease. Pancreatic fibrosis is the most frequently found alteration [1780] and is usually associated with interstitial inflammation, acinar and ductal dilatation [2586, 3228], and fat droplets in the pancreatic acinar cells [2625]. These changes, thought to be caused by protein deficiency, and the steatorrhea often seen in liver diseases have been explained by depressed secretin production in the duodenal mucosa under the influence of liver damage. Attempts to confirm this functional impairment experimentally or clinically by measurements of the serum

activity of pancreatic enzymes, such as amylase or lipase, or by studying the response to secretin have produced equivocal results. In cats with acute liver damage produced by chloroform, the serum-amylase level dropped significantly, but serum lipase decreased only slightly [2796]. In clinical liver disease, normal activity or an increase in the serum enzymes seems to be the rule. Hyperlipasemia has been found in cirrhosis [699], while normal lipase levels were reported in acute hepatitis [1897]. More recent studies with improved techniques have shown that levels of serum amylase and lipase are elevated in approximately one-fourth of the cases of cirrhosis and acute hepatitis [699]. No correlation was found between the enzyme levels and hepatic function, although significant elevation of the serum lipase probably is a bad prognostic sign. Volume, bicarbonate concentration, and enzyme activity in the duodenal juice after secretin injection are normal in acute or chronic liver disease even in the presence of steatorrhea [1295].

"Biliary Pancreatitis." Injection of bile under pressure into the pancreas causes edema and hemorrhagic pancreatitis [1596]. Intraluminal activation of trypsinogen does not cause pancreatitis unless some escapes from the lumen. Since simple reflux is not likely to cause this, the liver and biliary tract probably play a minor role in the pathogenesis of acute pancreatitis [2625].

Influence of the Pancreas on the Liver

Experimental Studies. Early animal experiments, which stimulated the concept of lipotropism (see Lipid Metabolism, Chap. 5), showed that fatty metamorphosis of the liver follows total pancreatectomy even if insulin is given [258]. This prompted Best to call the pancreas the "guardian of the liver." Exclusion of pancreatic juice from the intestine by ligation of the pancreatic duct has the same effect [599, 2333]. This fatty liver can be prevented by feeding choline or methionine but not by feeding intact protein [533]. Trypsin apparently is the anti-fatty liver substance of the pancreatic juice, and its mode of action is the proteolytic release of methionine [2332]. Pancreatic duct ligation in dogs causes an increase in serum-alkaline phosphatase activity, possibly as a result of the development of cholangiolitis [3030].

The Liver in Pancreatic Disease. In clinical pancreatic disease, several effects on the liver are seen. The common bile duct may be constricted or occluded by carcinoma in the head of the pan-

creas or, less commonly and less completely, by pancreatic cysts, especially cystadenomas, or by chronic inflammation and fibrosis in chronic relapsing pancreatitis [633, 2586]. This constriction or occlusion results in extrahepatic cholestasis.

Even in the absence of biliary obstruction, pancreatic disease, especially if associated with lithiasis, may lead to hepatomegaly, jaundice, and ascites [3120]. The most common histologic findings in the liver are fatty infiltration and fatty septal cirrhosis [887, 2884, 3024, 3120]. These conditions are seen in acute pancreatitis [2720], chronic relapsing pancreatitis [633, 2586], cystic fibrosis [133], and even carcinoma of the pancreas [2884], and the term "pancreaticohepatic syndrome" has been suggested [624]. The fatty liver and its sequelae may be explained by interference with hydrolysis and absorption of proteins, amino acids, fats, fat-soluble vitamins, and possibly choline. Toxic hepatic injury caused by release of pancreatic tissue-breakdown products into the portal blood probably aggravates the liver damage. Jaundice in chronic pancreatitis can be caused by concomitant choledocholithiasis, toxic injury to the liver, or mechanical compression of the common duct by the inflamed fibrosed pancreas.

Parallel Involvement of Pancreas and Liver

Hepatic Disease. Pancreatic necrosis in viral and toxic hepatitis is possibly the result of simultaneous injury to both organs. Hemochromatosis and vascular lesions, such as periarteritis nodosa, involve both organs simultaneously.

Alcoholism. Alcohol, in addition to producing hepatic changes, is also a factor in the pathogenesis of approximately half the instances of acute or chronic pancreatitis [2586]. Its effect on the pancreas may be a direct toxic one, the result of ductal obstruction because of duodenal congestion, or the result of reflux following persistent vomiting.

Malnutrition. The most important parallel changes in the liver and pancreas result from dietary, chiefly protein, deficiencies. Animals on a low-protein-high-fat diet develop acute interstitial pancreatitis with fat necrosis, followed by pancreatic atrophy, in addition to fatty and fibrotic changes in the liver [1106, 1300, 2013]. Similarly, pancreatic changes have been seen in malnourished persons in widely scattered areas all over the world [739, 1172, 1410, 3418, 3499]. Recently these changes have been produced in animals by administration of ethionine, the ethyl analogue

and metabolic antagonist of methionine [61, 977, 1200]. Continued administration of small amounts of ethionine for several weeks leads to chronic interstitial pancreatitis with pancreatic acinar atrophy [1825].

PATHOGENESIS. The pancreas is the organ which forms the greatest amount of protein per unit weight, most of which is enzyme protein [1094]. Dietary protein deficiency or conditioned amino acid deficiency quickly decreases the available enzyme protein precursors in the pancreas. This decrease is reflected in fatty infiltration and probably in decreased enzyme formation. The subsequent reduction of trypsin in the intestine de-

creases amino acid absorption and accentuates the fatty metamorphosis of the liver stimulated by the protein deficiency [1410]. In addition, increased demands for enzymes and insulin because of the high-carbohydrate diet usually eaten under such circumstances potentiates the effect of the low-protein diet [739]. Pancreatic alterations were noted before hepatic changes in some studies [739, 3418], but in others they appeared simultaneously [1106, 1172]. Pancreatic deficiency and its sequelae therefore seem to be aggravating factors tending to perpetuate the damage done to the liver and pancreas initially by protein or amino acid deficiency.

RELATION OF THE LIVER TO THE ENDOCRINE GLANDS

PITUITARY GLAND

In its role as the master gland, the pituitary influences many phases of hepatic metabolism. Early information was obtained by observing changes following hypophysectomy. Various pituitary extracts were then injected, but little clarification came until relatively pure fractions were available. Of these, ACTH has been the most extensively studied in both man and animals, and attempts have also been made to use it in the treatment of hepatic disease. The liver inactivates ACTH, although other organs may also do this [1156].

Hypophysectomy. STRUCTURAL CHANGES. Within two days following removal of the pituitary gland, hepatic glycogen is almost completely depleted, especially from the periphery of the lobule [753]. It returns to a nearly normal level after a month and again decreases after two months. The mitochondria swell and become spherical, and after one month the typical zonal distribution of these organelles is lost. At the end of two months they appear as abnormally fine granules scattered throughout the cell. The fat accumulates in the lobular center in the hepatic cells and in the Kupffer cells. Under some circumstances, possibly related to associated hypothalamic injury, such as in mongolism, this fatty metamorphosis becomes severe, leading to fibrosis and cirrhosis [534, 1240, 2806], although fibrosis also develops without preceding fatty metamorphosis [532].

FUNCTIONAL ALTERATIONS. Associated with the morphologic changes are (1) a decrease in gluconeogenesis, although in diabetic animals a low glucose uptake returns toward normal [2741]; (2) an increase in fatty acid synthesis [377] and inorganic phosphorus uptake by the liver [1157]; (3) a

decrease in hepatic pentose nucleic acid with no change in desoxypentose nucleic acid [485, 1157]. Some hepatic enzymes, such as *D*-amino acid dehydrogenase, appear to be depressed [1116], but this effect has been questioned [1641]. Hypophysectomy prevents fat accumulation following administration of adrenal extracts, and it prevents cirrhosis and carcinoma following azo dye administration, an effect not influenced by DOCA or cortisone but slowly counteracted by ACTH [2783].

Anterior Pituitary Extracts. Various anterior pituitary extracts have been given to normal and hypophysectomized animals. They act as a combination of ACTH and growth hormone, causing movement of depot fat as glycerides to the liver [258, 474, 3215], with an increase in the weight and in the hepatic content of lipids, water, and fat-free solids [472]. Hepatic oxygen consumption and acetoacetate production are increased [472, 938] with increased oxidation of endogenous fatty acids, a protein-sparing process [472].

Corticotropin (ACTH). Corticotropin decreases hepatic basophilia without change in hepatic phosphatase, especially if the animals are on a high-carbohydrate diet [139]. The administration of ACTH to normal animals decreases fatty acid synthesis [377, 3556] and produces a fatty liver [139, 1987, 1988] with peripheral predominance of fat. The fat-mobilizing agent is probably different from ACTH and other known hormones but is not well separated from ACTH [913, 2817]. Preparations can be made which cause a 40 per cent increase in liver lipids in 6 hours with no increase in phospholipids [667]. An intact adrenal gland is needed for this reaction [1589, 1967, 1968]. A fraction of adrenal extract which is not steroid in nature [1968] has been prepared which sustains the

ability of the adrenalectomized cat to deposit fat in the liver [1403]. This is probably a pituitary factor, most likely ACTH and some "triggering factors" from the adrenal gland [1968].

The livers of animals on high-carbohydrate diets contain much fat chemically and histologically after ACTH administration, while on high-protein or high-fat diets fat is not increased after ACTH administration even if the liver is fatty at the start [139]. On the other hand, the fatty liver which normally follows physical stress is prevented by the administration of sugar [1967].

EFFECTS OF CORTICOTROPIN IN MAN. Little is known about any direct effect of corticotropin on the normal human liver. It has been used in the treatment of acute hepatitis; while beneficial effects were reported at first [619, 2771], a more detailed study [959] showed (1) no enhancement of tissue repair; (2) frequent development of fatty liver; (3) poor circulating eosinophil response, not correlated with clinical results (viral hepatitis is not accompanied by eosinopenia, as are other virus diseases) [3542]; (4) increased incidence of hyperglycemia, especially glucosuria [619, 959, 1393], with depletion of hepatic glycogen stores [343]. However, symptomatic improvement is rapid and is associated with a prompt drop of the serum-bilirubin level [10, 959, 2771, 2895]. When administration of corticotropin is started early, the disease is more prolonged, relapses are more common, and the side reactions are numerous. When it is given later, Bromsulphalein retention returns to normal more rapidly. In cholangiolitis, complete recovery has followed ACTH therapy [2895]. In fulminant hepatitis, especially with hepatic coma, beneficial results from corticotropin treatment have been reported [855, 959].

Growth and Other Hormones. Administration of growth hormone to fed or hypophysectomized animals decreases liver fat. In fasted animals it increases liver fat, suggesting mobilization of depot fat followed by stimulated utilization, either for oxidation or for protein formation [1988]. The synthesis of acetoacetate is increased, and in this sense growth hormone is ketogenic [221]. Fatty acid synthesis is decreased [377], but phospholipid and nucleoprotein synthesis are increased [667]. Growth hormone also increases pentose nucleic acids in the liver, but the increased phosphorus turnover following hypophysectomy is reduced to normal by the hormone [1157]. Hepatic glycogen is decreased, and hypoglycemia

is found. In adult rats, but not in young males, urea formation by the liver, hepatic arginase, and alanine-glutamic acid transaminase are all reduced by growth hormone [197]. *d*-Amino acid dehydrogenase remains unchanged [1116]. In mice with large grafts of dependent pituitary tumors which secrete thyrotropic and gonad-stimulating hormones, hyperplasia and cystic dilatation of the extrahepatic bile ducts are seen [1111].

Posterior Pituitary Hormones. No direct effect of posterior pituitary hormones on the liver is known, but the action of pitressin, the antidiuretic hormone, is influenced by the liver. The liver is the most important site of inactivation of the hormone [962], and fatty livers or the livers of animals on a low-protein diet are not so efficient in this inactivation [282, 1393, 1960]. Antidiuretic hormone is increased in the urine in cirrhosis [2701], although the cirrhotic liver inactivates pitressin normally [1393, 2427, 3579]. Administration of pitressin to normal persons and patients with cirrhosis causes antidiuresis and hyponatremia, but in cirrhosis the effect is greater [1393].

ADRENAL GLAND

The liver is the target organ for much of the action of both cortical and medullary adrenal hormones. Early information was obtained following adrenalectomy or administration of rather crude cortical extracts, and it was difficult to ascribe specific morphologic and functional changes to individual hormones. The advent of purified hormones largely clarified this problem and also made possible many observations on their effects in man.

Adrenalectomy. Bilateral removal of the adrenal glands decreases hepatic glycogen [2028]. Carbohydrate metabolism proceeds normally in normal animals; but gluconeogenesis in diabetic animals is decreased [2741]. Adrenalectomy prevents fatty liver in rats intoxicated with carbon tetrachloride or phosphorus or fed a high-fat diet, even if salt is administered. It supposedly impairs deposition of fatty acids or neutral fat, since phospholipid turnover and fat absorption are normal [161]. However, phospholipid synthesis is probably depressed following adrenalectomy, because the plasma phospholipids and the phospholipid/protein ratio are reduced [3717]. Fatty acid utilization for oxidation and ketone body formation is reduced, and as a result, hepatic ketone body content is decreased [2028]. Various respira-

tory enzymes such as cytochrome [823], cytochrome oxidase [3340], and succinic dehydrogenase [358, 3340] are also reduced. Removal of the adrenal gland stimulates regeneration after partial hepatectomy if a high-protein diet is given [823, 998], while on normal or low-protein diets regeneration is depressed [241, 1987].

Adrenal Cortical Extracts. The metabolic effect of adrenal cortical extract in the liver is largely caused by its glucocorticoid content. Glycogenesis [406] and gluconeogenesis from protein [1819, 2054] are stimulated. Diabetes produced by alloxan does not prevent this response to adrenal extract in the adrenalectomized animal [2287]. Administration of cortical extracts after adrenalectomy increases or restores to normal the activities of many hepatic enzymes, including alkaline phosphatase [1816], cytochrome oxidase and succinic dehydrogenase [3340], and amino acid dehydrogenase [1641].

Cortisone in Animals. Cortisone is largely inactivated by the liver, primarily because of a change in the unsaturated ring structure [2071, 2947, 3596]. This inactivation is inhibited by para-aminobenzoic acid [3596]. Cortisone stimulates metabolism in general, and oxygen consumption and water production are increased [2028]. The hepatic cells enlarge because of an increase in cytoplasm [2562]. Hepatic glycogen is increased [1590, 2074], even if diabetes is produced.

Gluconeogenesis from glycine [1473] and protein [1819] is increased. Although body protein synthesis from dietary protein or nonprotein precursors is reduced, the rate of protein synthesis in the liver is stimulated by cortisone, and hepatic protein nitrogen and total nitrogen are increased [587]. In rabbits, doses of 10 mg cortisone per day cause hepatic necrosis [1152]. This also depresses regeneration following partial hepatectomy even if regeneration is stimulated by adrenalectomy [998]. Pentose and desoxypentose nucleic acids are decreased by cortisone, but the PNA/DNA ratio increases. Cortisone increases succinic dehydrogenase activity in the livers of normal rats and restores this enzyme to normal in adrenalectomized ones [358]. Hepatic alkaline phosphatase is also increased but not parallel to the degree of glycogen deposition [1816]. This effect is opposite to that of thyroxine and has been related to the gluconeogenesis from protein stimulated by cortisone [1819]. Epinephrine increases glycogen with no effect on phosphatase. Other enzymes, such as

arginase, remain unchanged after cortisone administration [1816]. Less is known of the effect on fat metabolism. Cortisone causes fatty livers in rabbits with lipemia [2750] and increases fatty metamorphosis in early carbon tetrachloride intoxication [116]. The block in fatty acid utilization following adrenalectomy is removed by cortisone [2783], and ketone body formation is increased [2028]. Cortisone inhibits hepatic fibrosis from carbon tetrachloride if the two substances are given simultaneously [115].

Cortisone in Man. NONHEPATIC DISEASES. The administration of cortisone to persons without liver disease produces few functional or structural changes referable to the liver. Fatty liver has been reported [3193], and slight and inconsistent changes have been demonstrated in various serum-lipid fractions [22, 1534]. Levels of serum protein and serum esterase sometimes drop [1534].

Hepatic glycogenesis from glucose is stimulated by cortisone, even in patients with gastric carcinoma, in which it is impaired and not influenced by insulin [3682].

HEPATIC DISEASES. In liver disease the response of the adrenals to ACTH is normal [343, 959, 1428], although the eosinophil response is depressed [2036]. In cirrhosis, decreased adrenocortical activity is suggested by increased lipids in the adrenal glands [2036] and increased neutral reducing lipids in the urine [3014]. Rare instances of cirrhosis have been seen in young women with the symptoms of Cushing's syndrome and very high serum-gamma globulin levels [343].

Cortisone has three principal effects in liver disease [3527]:

1. The psyche is stimulated, with increased appetite and better nutrition [1428].
2. Carbohydrate metabolism is altered, and liver glycogen is increased.
3. Fluid and electrolyte balances are changed.

Cortisone administration in acute hepatitis results in rapid regression of symptoms and an early loss of liver tenderness [959, 2771]. Jaundice and Bromsulphalein retention also subside faster [959, 1428]. No significant changes in the results of other hepatic tests are found, but histologically, recovery is more rapid after cortisone, despite a greater increase in fat, than with ACTH. However, the tendency for relapse is greater [959, 2771], and the disturbed carbohydrate metabolism is often manifested by hyperglycemia and glycosuria [959, 1428]. Water retention and severe mental

changes also occur [1428]. The incidence of chronic hepatitis is possibly increased [2771], or at least it is not prevented by the hormone [959]. Cortisone alone fails to help in hepatic coma [959], although it may be useful if combined with antibiotics [855]. In cirrhosis with jaundice and ascites, cortisone stimulates the appetite and increases food intake [3726]. This is associated with a prompt and dramatic rise of the serum albumin and cholesterol esters. Spider angiomas sometimes disappear, and, contrary to what happens in experimental animals, liver fat regresses [3726], although the liver enlarges rapidly and becomes very firm [548]. Cortisone also increases urinary volume and urinary sodium. Changes in the results of other hepatic tests are minor and transient [548].

The rate of disappearance from the blood of intravenously infused hydrocortisone in patients with liver disease is proportional to the degree of hepatic-cell damage [416].

Desoxycorticosterone and Aldosterone. The administration of desoxycorticosterone acetate (DOCA), a synthetic steroid influencing mineral metabolism, supposedly produces focal or central necrosis, fatty metamorphosis, hyperemia, hemorrhage, or pigment deposition in the liver of experimental animals [3080]. In the dog and rabbit, these changes are associated with an increase in number of plasma cells and eosinophils. In man, vacuolization of the hepatic-cell cytoplasm and disarray of the hepatic-cell plates have been reported [1055]. On the other hand, DOCA protects against liver damage produced by x-ray irradiation [915], and it is apparently a potent stimulus for hepatic protein formation and deposition [1096]. DOCA is inactivated by the liver if the cells are intact [2947]. Adrenalectomized rats treated with DOCA showed an unexplained increase in hepatic phospholipids [2285]. The natural mineral corticoid, aldosterone, probably is responsible for sodium retention in liver disease (see Excess Hormones, under Pathogenesis of Ascites, Chap. 29).

Epinephrine. Much of our knowledge of the effect of epinephrine on the liver comes from studies on unanesthetized persons, with the aid of catheters in the hepatic vein. Histologic and biochemical studies are sparse, but epinephrine apparently plays a role in hepatic metabolism other than its function in glycogenolysis, as evidenced by the depression of amino acid dehydrogenase following its administration [1641].

Epinephrine raises glucose concentration and decreases potassium in hepatic vein blood associ-

ated with a fall in hepatic glycogen [1489]. Hepatic blood flow, splanchnic oxygen consumption, and peripheral lactic acid are increased [195]. Norepinephrine has only a slight effect on any of these. Intraportal epinephrine causes intrahepatic vasoconstriction, especially in the smaller vessels, an effect similar to stimulation of the hepatic nerves in many species [717]. The glycemic response to epinephrine has been used as a measure of the amount of hepatic glycogen in man, but seasonal variations in the response and differences in epinephrine inactivation impair the validity of the procedure as a test [753]. In acute hepatitis the glycemic response is subnormal, possibly because of decreased glycogen in the liver [1489]. Young diabetic patients also have a subnormal response, whereas older diabetic persons behave normally. This fact has been explained on the basis of a less efficient mobilization of hepatic glycogen in young persons with diabetes mellitus (see Hepatic Glycogen in Diabetes, later in this chapter).

Urinary Excretion of 17-Ketosteroids and Corticosteroids in Liver Disease. Since the liver inactivates many hormones, less of their excretion products appears in the urine in liver damage. The urinary 17-ketosteroid excretion is low in all forms of liver disease, including extrahepatic biliary obstruction [343, 901, 1013, 1081, 1167, 2578, 2853], the depression being related to the severity of the disease. This diminished excretion is largely caused by a decrease in the glucuronate-conjugated fraction probably formed in the liver [343]. Some investigators have claimed that corticosteroid excretion is increased and that most of the increase is in the mineral steroid fraction, aldosterone, accounting for the very low urinary sodium excretion [343]. Others state that corticosteroid excretion is normal and proportional to that of creatinine in cirrhosis, with no diurnal variation [1209]. Recently the relation to creatinine excretion has been substantiated in normal persons, but not in cirrhosis, where the total corticosteroid excretion is normal or increased, with great day-to-day variations [2913]. The fraction of ketocorticosteroids having DOCA-like properties, probably aldosterone, is increased.

After administration of cortisone or other related steroids, the urinary level of 17-ketosteroids is low and corticosteroid excretion is increased in the presence of liver damage [449, 638, 2578, 2599]. Furthermore, in cirrhosis the amount of conjugated steroids in plasma is reduced [342]. This supports

the idea that these steroids are inactivated by conversion to 17-ketosteroids in the liver.

RELATION BETWEEN THE PANCREATIC ISLANDS AND THE LIVER

Insulin, manufactured in the islet cells of the pancreas, is released into the portal system and reaches the liver before any other organ. Insulin has been called the "central anabolic hormone" without which many of the building processes attributed to other organs could not proceed at the physiologic rate [257], and "the thermostat which regulates the level of homeostasis" [3139].

Action of the Liver on Insulin. Insulin is destroyed at the rate of 1/25 to 1/50 unit per kilogram per hour in the dog [3548]. This destruction is possibly carried out by the action of an enzyme, insulinase, which is present in the greatest concentration in the liver [2311].

Effect of Insulin on the Liver

Insulin influences carbohydrate, protein and fat metabolism, and growth in the liver in vivo and to some extent in vitro [257].

Carbohydrate Metabolism. Insulin acts largely on the glycogenic-glycogenolytic cycle in the liver. Originally insulin was thought to inhibit glycogenolysis and to increase glycogenesis in the liver [2053, 2652, 3139] when given in physiologic amounts. Insulin was also considered necessary for the action of hexokinase in the phosphorylation of glucose [661], either directly or as a counter-inhibiting factor of pituitary or adrenocortical hormones. Later experiments failed to substantiate this concept [3167], and studies on liver homogenates showed that hexokinase, phosphorylase, glucose-6-phosphatase, and phosphoglucomutase, the known enzyme systems, are not influenced by insulin or its absence [410]. When liver slices with intact cells from diabetic animals were incubated with tagged glucose, pyruvate, acetate, or formate, two blocks were found in the metabolism of glucose. The first is somewhere between glucose and fructose phosphate [559], although the change of fructose to glucose is not affected [560]. The second is in the incorporation of two carbon compounds into fatty acids. Oxidation of various intermediates, including fructose, to carbon dioxide proceeds normally in the absence of insulin [559, 992, 2499]. Insulin administration to the diabetic animal prior to sacrificing overcomes both blocks, while fructose administration

overcomes the second block and restores lipogenesis [142].

Since plasma phosphate is used in the formation of glucose-6-phosphate in the liver, the hepatic-cell surface has been suggested as the site of phosphorylation of hepatic glucose phosphate [2864], and insulin has been considered to be merely a factor necessary for the transport of glucose phosphate across the cell membrane [1973, 2312]. This explains discrepancies noted between experiments with liver slices and homogenates.

Insulin also has an effect on the glucose output by the liver. Normally in the rat, 14 to 15 mg per 100 gm per hour of glucose is added to hepatic vein blood [758]. In the normal man, insulin decreases the glucose output until the blood-sugar level falls. This stimulates the release of epinephrine, which increases the output [195]. In diabetes, the glucose output is increased to 21 mg per 100 gm per hour in the rat [758]; in man insulin also decreases the glucose output [2817].

Fat Metabolism. Insulin stimulates hepatic lipogenesis [304, 377, 556]. In the diabetic animal it does so only if given before the animal is killed [376]. A pituitary factor inhibits the insulin lipogenesis in the intact animal [377]. Insulin also prevents movement of depot fat and its accumulation in the liver, and it inhibits ketone body formation, thereby suppressing ketosis [257]. Phospholipid utilization appears normal in the absence of insulin [1387]. Synthesis is increased in both pancreatectomized and phlorhizinized dogs, possibly because phospholipid is an intermediary in fat oxidation, which is increased [796, 3714]. In the absence of insulin, cholesterol synthesis proceeds normally [376], but in alloxan-treated rats the serum-cholesterol level is elevated, in spite of normal liver lipids [268]. Cholesterol synthesis is increased in these animals when they are fed glucose, but it returns to normal if they are fed fructose [1549].

Protein Metabolism. Insulin accelerates the rate of protein synthesis [2069], and its absence causes failure of incorporation of amino acids into protein, while protein breakdown is not altered [1052, 1851]. In the alloxan-treated rat, hepatic desoxypentose nucleic acids, nuclear histone, and pentose nucleic acid are increased. This may be a direct effect of the poison [792]. Insulin decreases the glutamine and glutamic acid content of the liver [747], because deamination of glutamine to glutamic acid to alpha-ketoglutaric acid is increased. The alpha-ketoglutaric acid enters the Krebs cycle,

to be used either for oxidation or, more likely, for glycogenesis. This is prevented by glucose. The metabolism of other amino acids is not known to be altered.

Insulin Excess; Hypoglycemia. Excess insulin does not increase hepatic glycogen but may decrease it, probably because of the associated shock and reactive humoral mechanisms. Administration of insulin to rabbits decreases the liver glycogen in some animals which were able to withstand larger doses. When the glycogen had disappeared, these animals developed convulsions and died. Some animals, however, died early after small doses of insulin, and hepatic glycogen was increased, especially in the central zone. This glycogen was considered "locked in" by the insulin and not available for blood-glucose formation. In some rabbits, small scattered areas of focal necrosis were found after insulin shock [3294]. In man excess glycogen has been observed after hypoglycemic shock [2652].

In clinical liver disease, the response of the blood sugar to the administration of insulin in various tolerance tests is variable; it may be normal, exaggerated, or depressed [1494, 3461]. This is related not to hepatic glycogen content but apparently to variable enzyme and hormonal alterations [3724].

Glucagon

Glucagon, the pancreatic hyperglycemic factor, activates hepatic phosphorylase. This in turn releases glucose to the blood stream by breaking down hepatic glycogen. Glucagon acts only on hepatic cells, in contrast to epinephrine, and does not change the blood lactate or pyruvate levels [1740]. In contrast to insulin, glucagon not only increases the blood sugar but also stimulates the oxidation of fatty acids [1415]. Administration of the hormone followed by determination of the blood-sugar level has been suggested as a hepatic test [1740], especially if combined with epinephrine [3413].

Diabetes Mellitus and the Liver

Some of the effects of diabetes mellitus on the liver are well established, while others remain the topic of much controversy. The most debated issue is glycogen content of the hepatic-cell cytoplasm.

Hepatic Glycogen in Diabetes. Four factors are mainly responsible for the contradictions about the glycogen content of the diabetic liver: (1) species differences in experimental animals and man; (2)

differences in the experimental approach; (3) the effect of previous treatment; (4) post-mortem glycolysis. Hepatic glycogen stores are depleted in the dog liver following pancreatectomy, in alloxan-treated rats in ketosis and coma [1210, 1686, 3371], and in some instances of human diabetic coma [341]. This led to the claim that hepatic glycogen depletion is a characteristic finding in diabetes [1839] and that this is the chief cause of ketosis and coma [2310]. Recent experimental and clinical experiences have challenged the significance of these findings, and a more complex relationship between diabetes mellitus and hepatic glycogen has become apparent.

EXPERIMENTAL DIABETES. During acute alloxan diabetogenesis, liver glycogen changes, contrary to the blood sugar [697]. Alloxan probably not only destroys the beta cells of the pancreas but has other actions [697, 3402], such as inhibition of glycogenolysis, as demonstrated in the perfused liver [1211]. In some instances the initial hypoglycemia is not corrected by glucose administration and death results, with necrosis being found in the liver. This suggested that part of the hypoglycemic phase results from liver damage [693] and that part may result from the action of glucagon.

In established alloxan diabetes in the fasted animal with hyperglycemia or a normal blood-sugar level, hepatic glycogen is increased [697], and it is maintained better than normal [3524]. In some instances in the alloxanized rat and rabbit hepatic glycogen is normal [1381]. If hypoglycemia is present, liver glycogen is depleted. No correlation is noted between the glycogen in the liver or muscle or between the fasting blood sugar and ketonuria [697]. Only terminally does the hepatic glycogen level drop [1686]. Cortisone-induced diabetes in animals is also associated with very large amounts of glycogen in the liver [965, 1590].

CLINICAL DIABETES. In some instances of clinical diabetes mellitus, hepatic glycogen is normal. In others it is even increased, chemically as well as histologically, in necropsy [2652, 3402] or biopsy specimens [341, 1489, 1856]. The increased glycogen refers to cytoplasmic depositions (Fig. 204, lower), not to nuclear glycogen (Figs. 6, lower right, 204, upper), which is common in all types of human diabetes. Excess glycogen sometimes accounts for the enlargement of the liver in diabetes. This is found even in some patients dying in diabetic coma, while in others it is absent. Some of the findings are difficult to evaluate because of previous therapy. The increased hepatic glycogen has

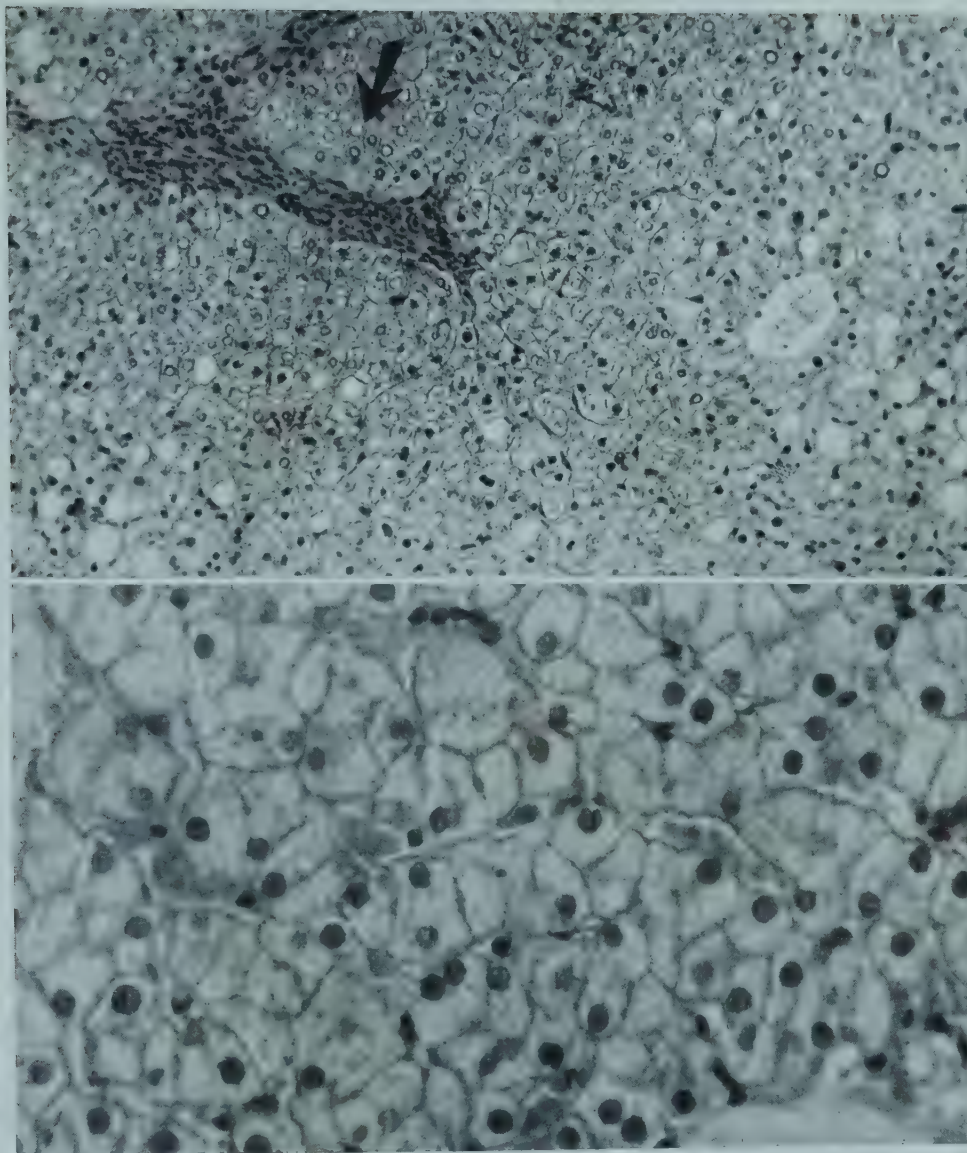


FIG. 204 Liver biopsy specimens of patients with diabetes mellitus. *Upper.* Slight fatty metamorphosis in central zone and nuclei rich in glycogen on the lobular periphery (arrow) ($\times 110$). *Lower.* Hepatic-cell cytoplasm rich in glycogen and therefore fine-vacuolated in paraffin section ($\times 330$).

been explained by excess gluconeogenesis from fat in the liver. It points to the difference between the diabetes in man and in the pancreatectomized animal [3402]. Different types of diabetes have been suggested in man [341, 522, 3299], differentiation being based largely on the sensitivity of the hepatic glycogen to insulin [195]. In the hepatic sensitive type, the blood-sugar level is more sensitive to insulin than normal. This supposedly occurs in young people, who readily go into ketosis. Liver tissue obtained at biopsy appears normal, with a normal glycogen content. In the hepatic insensitive type, the blood-sugar response to insulin is less than normal; this occurs in middle-aged, generally obese diabetic patients, who have fatty livers with some decrease in glycogen. In ketosis, a previously

sensitive type becomes insensitive, although the liver biopsy may show no change.

Hepatic Dysfunction in Diabetes. The incidence of functional abnormalities varies as much as that of structural changes. In one series in which over half the patients were in acidosis, no consistent evidence of hepatic dysfunction was found [415]. When present, it is transient and mild and usually varies with the control of the diabetes [1067, 1261]. Age, sex, and the duration of the disease are of no significance [1937]. Bromsulphalein retention is the most common abnormality, reported in almost half the patients [1937, 2622, 3722], and is related to the severity and complications of the diabetes [2622] and the amount of fat in the liver [3722]. Abnormalities in the various flocculation

tests and the serum cholesterol are present in one-fourth to one-fifth of the cases, with the exception of the colloidal gold test, which is apparently more sensitive (Table 62). A similar number have an

Table 62 Percentage of Abnormal Results of Various Hepatic Tests in Diabetes Mellitus

Test	No. cases	% abnormal	References
Bromsulphalein	570	42	2622, 1937, 3722
Colloidal gold	247	37	1261
Thymol turbidity . . .	348	26	2622, 1495, 3722
Cephalin flocculation	473	25	1937, 1495, 3722
Serum cholesterol . . .	445	20	1937, 1495
Zinc sulfate turbidity	255	19	2622
Colloidal red	255	19	2622
Bilirubin	473	8	1937, 1495, 3722

altered A/G ratio [1937]. Carotenemia is found in some, although vitamin A levels are usually normal [1495]. Hyperbilirubinemia is found in less than 10 per cent of diabetic patients, and clinical jaundice caused by diabetes is rare. Urobilinogenuria occurs as frequently as hyperbilirubinemia. Serum-lipid levels are elevated in almost half the patients, but this is related to factors other than primary hepatic dysfunction [2622].

In the group of patients with hyperglycemia and glycosuria caused by hepatic disease [1935, 1937], treatment of the liver disease without insulin improved the hyperglycemia [1937]. Conversely in patients with hepatic dysfunction associated with diabetes mellitus, regulation of the diabetes is followed by improvement in hepatic function.

Changes in Hepatic Structure in Diabetes. The livers of diabetic patients are palpable in less than half the cases [1495, 3722], and in adults, enlargement is only slight [1067, 1380]. Also, at necropsy the livers are only slightly heavier than normal. Estimation of size by palpation is not well correlated with the size at autopsy and is not at all correlated with the body weight [2735]. In juvenile diabetes, hepatomegaly is common, usually because of fatty metamorphosis [3490] and sometimes because of increased glycogen.

MACROSCOPIC APPEARANCE. The morphologic appearance of the liver in diabetes depends upon

the control of the disease. In uncontrolled diabetes, the liver is enlarged. It has a rosy color when the glycogen content is high. It is often edematous. It frequently shows fatty metamorphosis, especially in children.

HISTOLOGIC FINDINGS. The most characteristic feature of diabetes, although not a specific one, is glycogen in the nuclei of the hepatic cells in the form of large vacuoles [571] (Figs. 6, lower right, 204, upper) (see Glycogen, under Cytoplasm, Chap. 3). The etiology and nature of this accumulation are unknown, but it appears to be related to the blood-sugar level. The Kupffer cells are frequently enlarged and contain fat droplets with strong vitamin A fluorescence. In autopsy specimens this bright fluorescence often stands out in contrast to the absence of fluorescence in the hepatic cells [2625] (Fig. 18). Collagenous membranes frequently enforce the reticulum network ("sclerosis of the liver") [2797]. Increased cytoplasmic glycogen produces fine vacuolization in paraffin sections (Fig. 204, lower), in contrast to the larger droplets produced by fat (Fig. 204, upper). Special stains may be needed to make this difference clear.

FATTY LIVER—CIRRHOSIS SYNDROME IN DIABETES. In some series, fatty liver was found in over half the cases examined and was even considered a major factor in the deaths of several patients [1616, 3490]. In others, fatty liver was present at death in less than 20 per cent of patients [2614, 2779]. With acidosis and coma fatty liver is common; if death results from other causes, fatty liver is not more frequent than in nondiabetic patients, which may explain some of the discrepancy [2735]. In biopsy specimens, similar variations in the incidence of fatty metamorphosis have been reported [415, 1937, 3722]. The amount of fat has been well correlated with insulin insensitivity and less so with age, obesity, and the duration of the disease [3722]. Hemosiderin pigment is frequently found in these fatty livers [3722].

Cirrhosis is an uncommon complication, having been found in 0.44 per cent of over 20,000 diabetic patients [1665]. If it is present, it is explained by other causes, such as alcoholism, hepatitis, or cardiac failure [1067]. However, cirrhosis apparently is more common in the diabetic than in the nondiabetic person [640, 1616, 2614, 2779], although this has also been questioned [2735]. Cirrhosis has been found in diabetic patients in biopsy specimens [1937, 3722], but too few cases are available to determine its incidence.

HEPATITIS. Homologous serum hepatitis epidemics have been reported in diabetic patients in clinics [844] and hospitals [3047]. This has been largely eliminated through proper sterilization and use of syringes.

GALLBLADDER DISEASE. The hypercholesteremia often associated with diabetes is a predisposing factor in the formation of gallstones. This accounts for the increased incidence of cholelithiasis in diabetes—31 per cent as compared with 21 per cent in nondiabetic persons [3490]. On the other hand, gallbladder disease may be a factor in the production or aggravation of diabetes because of the frequently associated pancreatitis [3380] (see "Biliary Pancreatitis," under Influence of the Liver on the Pancreas, Chap. 61), although well-substantiated instances of this are unusual [3490].

RELATION BETWEEN THE LIVER AND SEX ORGANS

Changes in hepatic function alter the structure and function of the primary sex organs, as well as the action of their secretion products, which in turn affects the secondary sex organs. Moreover, the function and structure of the liver seem to be altered by variations in the hormonal status, although here the evidence is less complete. The effect of liver disorders upon the primary as well as the secondary sex organs is largely determined by the fact that the liver inactivates, or "detoxifies," several of the sex hormones and fails to do so when it is diseased.

Effect of the Liver on Estrogens

The effect of liver on estrogens has been established in experimental animals and in man by several observations, not necessarily related to one another.

1. Estrogens are inactivated in vitro by liver tissue of various animals [935, 1448] and of man [3289, 3381]. Homogenates are as effective as slices, although single fractions separated by centrifugation are inactive [2768]. Stilbesterol, however, is destroyed by the microsomal fraction and also by the supernatant layer containing riboflavin or by riboflavin monophosphate alone. The livers of animals castrated or treated with estrone are less effective for in vitro inactivation [2854]. Diphosphopyridine nucleotide (DPN) is necessary for the process [657], and the end products are unknown [1999].

2. Estrogen inactivation is accomplished by

heart-lung-liver preparations; heart-lung preparations are ineffective.

3. In some animals, ovaries or estrogen pellets implanted in the spleen exert little or no estrogenic effect [284, 2987]. In the monkey [1541] and in man [3381] this can not be clearly shown.

4. The effect of endogenous or exogenous estrogens is enhanced in animals or man following liver damage by carbon tetrachloride [3292], partial hepatectomy [2551], nutritional deficiency [285, 3052, 3393], cirrhosis [1541], viral hepatitis [3728], or hepatic carcinoma [251]. This effect can also be inferred from the rise in titer of the circulating estrogens in liver damage [2551]. Despite reduced urinary gonadotrophins, which the liver also supposedly inactivates [1347], in all types of liver disease, estrogen excretion is increased [2599]. The proportion of unconjugated or active estrogen is often increased in cirrhosis [220] or moderately severe hepatitis [1167], although the total estrogens may be normal. Exogenous estrone increases both the total and unconjugated urinary estrogen excretion in severe hepatitis.

5. Ovariectomized rats on high-fat diets develop permanent estrus with estrogens in the urine. This does not occur on normal diets.

6. The highest concentration of estrogen in the body is found in the liver. Also, partial hepatectomy interferes with the conversion of estrone to estriol, and estrone is excreted in the urine unchanged [2923].

7. Impaired estrogen inactivation by the liver has been said to be caused by vitamin B complex deficiency [285], particularly thiamine and riboflavin [2988]. However, malnutrition, especially protein deficiency, seems to be more important [842, 1629]. Methionine appears to be the most potent substance in the treatment and prevention of malnutrition hyperestrogenism, and the effectiveness of vitamin B complex has been challenged [1318, 1629]. On deficient diets, inability to inactivate estrogen occurs before evidence of necrosis or fibrosis [1629]. Cystine and choline restore estrogen inactivation, but vitamin E does not.

Observations which speak against an effect of the liver on estrogens are the normal estradiol metabolism in carbon tetrachloride intoxication [154] and the normal estrogen content of the liver in human cirrhosis [3312].

Metabolic Pathway of Estrogen Inactivation. If estradiol is given intravenously in large doses, much appears in the gallbladder [154] or in the

bile of bile fistula dogs [2551]. Estrogen is conjugated in the liver with glucuronate or sulfate, and some of this inactive material is returned to the blood. Five to ten per cent of the total is excreted in the urine [2599]. Estrogen in the bile has been reactivated apparently during the process of biliary excretion [480, 2550, 2781]. Even exogenous estrone is partly converted to the more active estradiol [2551]. After excretion, high concentrations of estrogens are found in the portal vein, suggesting an enterohepatic circulation [480, 2550, 2781].

Clinical Hyperestrogenism. Inadequate neutralization of estrogens in liver disease results in hyperestrogenism. Its clinical signs are common in acute or chronic human liver disease and appear as structural or functional changes of the gonads and secondary sex organs, or in the skin and hair distribution.

CHANGES IN GONADS. Testicular atrophy is often found in chronic hepatic disease [852, 2036, 2351], unrelated to the age of the patients [220] but associated with active disease [2714]. The main alteration is thickening and fibrosis of the basement membrane of the tubules [2714], with decreased spermatogenesis. These changes are reversible to a large measure when the activity of the hepatic disease subsides [220]. They have been explained by vitamin E deficiency because of poor intestinal absorption owing to lack of bile salts. These changes are associated with impotence and loss of libido [191, 943, 2036] in up to 70 per cent of patients with cirrhosis. Menstrual irregularities are common in women with liver disease [191, 2036, 2719].

CHANGES IN THE PROSTATE GLAND. Prostatic alterations are common, such as delayed involution, and squamous metaplasia of the glandular or ductular epithelium, particularly in the utriculus, the remnant of the Müllerian duct [220, 2036, 3257, 3662]. Benign prostatic hypertrophy is less common, but carcinoma of the prostate occurs with the same incidence in men with or without cirrhosis [1644].

UTERINE CARCINOMA. Hyperestrogenism caused by impaired hepatic metabolic function has been suggested as a cause or a factor in the formation of carcinoma of the uterus [128, 3147], but this has been denied [3289].

GYNECOMASTIA. Gynecomastia occurs in about 40 per cent of men with chronic hepatic disease [220, 1477, 1615, 1793, 2036, 2393, 3568], either primary or secondary to malnutrition and refeed-

ing [1693] or to other diseases, such as ulcerative colitis [1891]. Two types are noted: the first is the result of insufficient inactivation of estrogen by the damaged liver; the second, seen in repatriated prisoners of war during refeeding or in cirrhosis during treatment [1113, 1693], is probably caused by restoration of estrogen production prior to recovery of estrogen-inactivating ability [1693]. The triad of cirrhosis with gynecomastia and testicular atrophy has been called the Silvestrini-Corda syndrome [2036]. Whether breast changes occur in women with cirrhosis is not established.

CHANGES IN HAIR DISTRIBUTION. Excess estrogen is held responsible for the characteristic hair distribution in men with cirrhosis, seen to some extent in 85 per cent of cases [943, 2036]. Hair on the chest is missing ("pectoral alopecia"). Hair does not extend from the mons pubis to the navel, and a sharp horizontal line limits the pubic hair, giving the impression of a female escutcheon. The axillary hair is reduced or missing. This is also noted in women with cirrhosis. These changes are part of the Chvostek habitus. Chvostek thought that these changes resulted from a congenital female preponderance in the male and that they were responsible for cirrhosis. They are probably secondary to cirrhosis. Clinical observations have not clearly established that the hair distribution actually changes during the course of cirrhosis. In exceptional cases in women, masculinization with hirsutism occurs, indicating the complexity of the endocrine changes and suggesting a compensatory mechanism mediated through the adrenal or pituitary gland.

SPIDER NEVI. Telangiectatic hemangiomas, variously called "vascular spiders," "spider telangiectasis," "spider angiomas" or "nevi," "stellate nevi," "spider webs," or "spider cancers," are considered to result from excess circulating estrogens [191, 2535]. They are common in pregnancy, disappearing after parturition, and in liver disease, where they were formerly thought to imply a bad prognosis. They have been compared with esophageal varices [398]. Spider nevi have a bright red central core, which is the size of a pinhead and is usually just barely palpable, although some are larger and more elevated. Fine hairlike branches radiate from this for about a centimeter. At times only these fine vessels are seen, in both the skin and mucous membranes, like silk threads in paper money ("paper money skin") [193]. Two types of spider are seen histologically. The first is arterial, with arteriolar branches showing many ramifications

The second looks like the arterial segment of an arteriovenous anastomosis with glomus formation. Spiders occur mainly on the face, upper trunk, arms, and fingers. Few appear on the lower trunk or legs, and rarely are they seen on mucous membranes, especially of the nose and mouth. They have also been found in the stomach, intestine, and genitourinary tract. Blood flows from the center outward, and pencil-point pressure applied to the core will cause the spider to disappear. Pulsations can be seen if gentle pressure is applied with a cover slip. The pressure in the vessels is less than systolic but higher than the venous pressure. Epinephrine first dilates, then contracts the vessels, while histamine dilates them.

PALMAR ERYTHEMA. Palmar erythema is noted on the hypothenar and thenar eminences of the palms and on the soles in liver diseases; it is sometimes referred to as "liver palms" [191, 2561]. The degree of erythema varies somewhat with the condition of the patient and with the number of spider nevi. It is supposedly caused by excess circulating estrogen and can be brought out by estrogen administration [191]. The clubbing of the fingers occasionally seen in chronic liver disease, especially in biliary cirrhosis, may also be related to excess circulating estrogen [243].

Influence of the Liver on Corpus Luteum Hormones

Free pregnanediol and pregnanediolone are inactivated by the liver [1249, 3004]. They are not stored in the liver or excreted in bile [1924].

Influence of the Liver on Testosterone Metabolism

Testosterone is metabolized by the liver differently in various species [2876, 3274]. In birds and mammals two mutually depressing systems requiring citrate or diphosphopyridine nucleotide (DPN) are present. DPN converts the alcohol form of the hormone to a ketone forming a 17-ketosteroid, while the citrate system changes the conjugation in the ring structure. In fish, amphibians, and reptiles, a double bond is destroyed by enzymes in the liver not requiring citrate or DPN, and 17-ketosteroids are not formed. In hepatectomized animals the inactivation of testosterone is decreased [3564].

Influence of Sex Hormones on the Liver

Many contradictory observations have been reported concerning the effects of the sex hormones upon liver function and structure.

Castration. Castration causes no significant change in liver weight [1923], but it does cause shrinkage of the size of the lobules [1354] with decreases in basophilic granulation [1840] and in the number of binucleate cells [1448]. Serum globulin is increased, while albumin drops [1923]. The tolerance for quick-acting barbiturates is reduced, suggesting that hormones are cofactors in the process of detoxification [466].

Testosterone Administration. The chief effects of testosterone are anabolic and therefore protein-sparing. Because of this it has been ascribed a protective effect on the liver. It reverses many of the changes caused by castration. The liver weight of castrated male rats is increased [1923], basophilic granulation is restored [1840], and hepatic alkaline phosphatase activity is slightly increased, with no change in arginase [1818]. Changes in the serum proteins are prevented [1923], and the lowered tolerance to quick-acting barbiturates is restored to normal [466]. Testosterone exerts little effect in normal animals. Liver weight is influenced in some species [293, 3004] but not in others [1923]. In liver homogenates of normal female rats, testosterone inhibits citrate synthesis [852].

Clinically, testosterone does not change the results of the hepatic tests in liver disease, but it produces subjective improvement in the patient [2810]. In chronic hepatitis and cirrhosis, it has an anabolic or protein-sparing effect [1113, 1767], but a good diet is more effective in restoring a positive nitrogen balance [1113]. Jaundice caused by methyltestosterone has been discussed (see Methyltestosterone Jaundice, Chap. 41).

Estrogen Administration. Administration of progesterone or estrogen to castrated females restores the number of binucleate cells to normal [40]. Estrogen administration has been claimed to be harmful to the liver, but no convincing evidence is found in results of hepatic tests in man [1086] or in histologic studies in animals [1252]. Estrogen administration to mice causes hyperplasia and hypertrophy of the bile ducts, even with cystic dilatation [1131], and in experimental granulosa cell tumors in mice, cavernous dilatation of the sinusoids occurs, followed by thrombosis and scar formation [1110].

Synthetic estrogens, such as stilbesterol, inhibit succinoxidase and other oxidative enzyme systems [2162], but this is not related to estrogenic potency [511].

Effect of Sex Hormones upon Hepatic Fat Deposition. The effect of sex hormones upon he-

patic fat deposition is confusing. More hepatic fat accumulates in female rats than in males immediately following hepatectomy, and ovariectomy decreases this [3288].

On choline-deficient, high-fat-low-protein diets, male rats develop fatty livers before female rats, and testosterone aggravates the effect in young female and adult male rats [929]. (Administration of estrogens decreases fat deposition, and a lipotropic effect has been ascribed to them in that they are supposed to improve methionine utilization [1322, 1324, 2285]. Ethyl estradiol is more effective than estradiol benzoate or estrone [1324]. The lipotropic effect of estrogen may not be direct but may rather be the result of a depression of a pituitary effect on fat storage [929, 3051].

Under some circumstances, testosterone exerts a lipotropic effect. For instance, female rats are more

susceptible to the development of fatty liver in acute ethionine intoxication than male rats [975]. Males become susceptible after castration, and both females and castrated males can be protected by testosterone. In view of the antagonism of ethionine to methionine, this effect has been considered the result of protein-sparing action.

In conclusion, the contradictory lipotropic effect of the sex hormones probably depends on the efficiency of pituitary stimulation and sulfhydryl utilization.

Sex Differences. Significant structural sex differences in the liver do not exist in man. Differences in growth are noted, as in any organ. For instance, during growth, the ratio of hepatic cytoplasm/nucleus increases in males, in contrast to females, where it is constant, partly because of the anabolic effect of androgens [477].

RELATION OF LIVER TO KIDNEY, CARDIOVASCULAR SYSTEM, SKELETON, AND CENTRAL NERVOUS SYSTEM

HEPATORENAL RELATIONSHIPS

The relations between the liver and kidney depend upon their common excretory, metabolic, and vasoregulatory functions and upon their common susceptibilities to injurious factors. Therefore, damage to either organ influences the function and structure of the other. The vague term "hepatorenal syndrome" has been used by different authors to connote different associations.

Common Functions

Excretory Functions. Many dyes are excreted in the urine and bile. The chemical structure of the dye determines the preferential route of excretion [2245]. Dyes such as Bromsulphalein [1587, 2454] or azorubin S, which are not normally excreted by the kidney, appear in the urine if hepatic damage interferes with biliary excretion. This is also true of Cholografin, used in intravenous cholangiography, which, in liver damage, may be excreted in high enough concentrations in the urine to produce a pyelogram. Many other substances, such as urea, appear in the bile in increased amounts in renal insufficiency. This increase is not only caused by the simple accumulation of substances in the blood in renal insufficiency but is actually compensatory excretion in the bile. The excretion of urobilinogen and bilirubin has been discussed (see Bilirubin, Chap. 11).

Metabolic Functions. The kidney is engaged in gluconeogenesis [3139] and in conjugation of benzoic acid to hippuric acid in some species, although in both instances to a lesser degree than the liver. The kidney does not participate in urea or serum-protein formation. Both organs are important in acid-base and water balance.

Vasoregulatory Functions. The relation between liver injury and vasoregulation is confusing. Evi-

dence has been presented for both a vasodilator and a vasoconstrictor effect of liver damage. A balance between a renal vasoexcitor material (VEM) and a hepatic vasodepressor material (VDM) supposedly determines the type of blood flow through the capillaries and the number of open capillaries [3057]. The hepatic vasodilatory principle slows the blood flow through the capillaries and increases tissue hydration. The hepatic principle has been identified as iron-containing ferritin or iron-free apoferritin [131, 2252] (see Ferritin, under Plasma Proteins, Chap. 6). The structure of the antagonist in the kidney, VEM, is not so well known. Both appear to be formed in increased amounts in anoxia, as in hemorrhagic shock [3057]. In prolonged and more drastic hypotension, the renal blood flow is abolished and VEM release ceases, whereas VDM formation continues. The prolonged VDM release produces irreversible vaso-depression. The balance of VDM and VEM possibly is disturbed in experimental and human hypertension, in which both factors are found in increased amounts [3056]. In liver diseases and in experimental cirrhosis, VDM is increased and VEM reduced, the latter possibly because of protein deficiency [3055]. This increased release of VDM from the liver predisposes to edema formation, because VDM is antidiuretic, one factor possibly responsible for water retention in liver damage. In patients with cirrhosis and antidiuresis, VDM is increased in the blood [131]. The vulnerability to injury of cirrhotic rats has been explained by a preshock stage caused by excess VDM [3055].

Normal hepatic cells possibly destroy vasoconstricting agents, and in hepatic injury excess of such agents may produce renal injury.

HYPERTENSION IN LIVER DISEASES. In liver diseases essential hypertension usually disappears, while neurogenic and renal hypertension are not

prevented [2081]. The liver has been said to play a role in the cause and perpetuation of hypertension.

In human cirrhosis, the incidence of hypertension is significantly lower than in noncirrhotic persons, possibly owing to excess circulating VDM [3146, 3290].

Common Injuries

Many protoplasmic poisons damage the hepatic cells simultaneously with the epithelium of the renal tubules, especially the proximal convoluted tubules [2480]. The anatomic changes in the epithelial cells in both organs are astonishingly parallel; for instance, carbon tetrachloride produces fatty and necrotizing changes in both. The toxic hepatic changes and acute nephrosis following burns are similar parallel injuries [362]. Diseases such as septicemia, yellow fever, Weil's disease, and eclampsia [370], and the Waterhouse-Friderichsen syndrome [2214], affect the liver and the kidney. The combined damage in diphtheria has elicited the term "hepatonephrite" in the French literature. The hemorrhagic renal changes in lipotropic deficiency are an example of parallel metabolic injury which occurs in choline deficiency in weaning rats [1281, 1453], in protein deficiency [1320], and in ethionine intoxication [3446]. Shock also produces lesions in both organs, the lesions differing because of characteristic blood supply of the kidney and liver. The central hepatic necrosis of shock is associated with segmental destruction of scattered parts of the nephron characteristic of acute nephrosis [2480]. Glomerulonephritis is increased in frequency in cirrhosis [2536].

Influence of the Liver on the Kidney

Renal Structural Changes in Experimental Animals. Renal structural alterations may be directly and independently produced by the same injurious agent which causes hepatic lesions, for example by allyl formate or carbon tetrachloride intoxication. In contrast, experimental biliary obstruction produces insignificant histologic changes in cats even after biliary decompression [481]. They become more apparent if dehydration is present [309], or if the renal artery and vein are obstructed [3497]. Temporary occlusion of the hepatic vasculature alone produces glomerular and tubular changes.

Renal Structural Changes in Man

Five morphologic lesions occur frequently in hepatic disease, especially with jaundice, if coexist-

ing glomerulonephritis and cirrhosis are omitted [2536]: (1) toxic glomerulitis; (2) fatty metamorphosis of the convoluted tubules; (3) biliary nephrosis; (4) acute segmental nephrosis, previously called "lower nephron nephrosis"; (5) necrotizing nephrosis. Only biliary nephrosis occurs exclusively in liver disease. The others are nonspecific changes occurring in many different conditions.

Toxic Glomerulitis. A mild toxic glomerulitis with increased permeability of the glomerular loops for protein is a common nonspecific finding in any severe hepatic disease. This lesion is found in various intoxications and infections and may only reflect anoxia. It is responsible for albuminuria and casts but not for any other impairment of renal function.

Fatty Metamorphosis of the Proximal Convoluted Tubules. Fine droplets of fat on the base of the epithelial cells of the proximal convoluted tubules are seen in fulminant hepatitis [2085] and possibly represent excretion of hepatic-cell-breakdown products. This has no functional significance.

Biliary Nephrosis. In the jaundiced patient, the epithelium of Bowman's capsule and of the proximal convoluted tubules contains bile pigment granules, and pigment casts appear in the tubular lumen, particularly in the lower nephron. This is followed by degenerative and even necrotizing changes of the pigmented epithelial cells, especially near casts. Vacuoles and hyaline droplets are frequently seen in the epithelial cells, and evidence of regeneration is noted. The basement membrane is intact, and involvement of glomeruli is not part of the process. This impressive morphologic picture has been called "cholemic nephrosis" or "biliary nephrosis." It is apparently a direct effect of bile pigment filtered through the glomerulus into the tubular lumen, concentrated and probably reabsorbed from there. Bile acids possibly play a role, although biliary nephrosis is best correlated with the excess prompt-reacting bilirubin in the blood and its excretion in the urine.

Biliary nephrosis is not found in hemolytic jaundice, in which bilirubinuria does not occur. In this condition bilirubin crystals are deposited in the interstitial tissue and epithelium of the renal papilla ("bilirubin infarct of the newborn"). It is not severe in fulminant viral hepatitis, in which the urinary bilirubin excretion is low. It is conspicuous in cholestasis of any type. Biliary nephrosis is the renal lesion best correlated with the degree and duration of jaundice. Urinary bile pigments do not seem to cause renal functional dis-

turbances. Bile casts can be experimentally produced in dehydrated animals with ligated bile ducts [309, 1578], just as heme casts can be produced in similar animals by injecting hemoglobin [1902]. However, no evidence is available that they produce other than local effects.

The morphologically impressive finding of a dark-green kidney has little functional significance. Clinically, it does not cause renal insufficiency and is noted chiefly by the presence of green pigment, casts, and pigment granule-containing epithelial cells in the urine.

Acute Segmental Nephrosis. The functionally most significant lesion is acute nephrosis, which occurs in many conditions associated with shock. It is characterized by necrosis of segments of the nephron, associated with breaks in the tubular basement membrane and with interstitial edema, inflammation, and even granuloma formation. This lesion develops in all parts of the kidney but predominantly at the corticomedullary junction, which gave rise to the name "lower nephron nephrosis." The term "acute nephrosis" is preferable, however, since other parts of the nephron are also involved. Some even use the term "glomerulonephrosis" to indicate the simultaneous involvement of the glomerulus [1093]. This nonspecific renal lesion accounts for most of the renal functional changes in liver disease, such as azotemia and oliguria. It is responsible for "urémie hépatique," which is a complication of severe hepatic failure and coma, finally causing the death of the patient. A rising BUN level is an ominous prognostic sign in biliary obstruction or cirrhosis [2639].

A much milder lesion characterized by combined spotty alterations of both glomeruli and tubules has been called "glomerulotubular nephrosis." Such a lesion occurs in many hepatic alterations, with or without necrosis. Its extent is supposedly well correlated with the hepatic lesion [1093]. It represents a precursor of the acute nephrosis and is apparently not yet associated with renal insufficiency. It has been explained by spotty vasoconstriction in the kidney caused by circulating substances otherwise destroyed by the normal liver.

Acute nephrosis is best explained as the result of ischemia of segments of the nephrons caused by temporary contractions of arterioles in shock [2480]. The contractions are enhanced by lack of neutralization of antidiuretic hormones (see Posterior Pituitary Hormones, under Pituitary Gland, Chap. 62). The shock stage may be caused by

disturbances of vasodepressor mechanism (VDM) (see Vasoregulatory Functions, earlier in this chapter), by dehydration, by disturbances of mineral metabolism, and by anoxia. Shock and renal segmental vasoconstriction are probably far more important causes of renal failure in liver disease than toxic hepatocellular breakdown products or intestinal toxins not neutralized by the damaged liver.

Necrotizing Nephrosis. Necrosis of continuous portions of the nephron, especially the entire proximal convoluted tubule, best designated as necrotizing nephrosis, is caused by various toxins or poisons, such as carbon tetrachloride [2480] or diethylene glycol [3097], which injure the kidney and liver simultaneously.

Renal Lesions in Various Hepatic Diseases. The renal lesions described occur singly or in combination. In fulminant hepatitis, toxic glomerulitis and fatty metamorphosis occur, while in more protracted hepatitis, biliary nephrosis is more common [2085]. This is sometimes associated with acute nephrosis, depending upon the presence of shock in the terminal period. The same holds true for fatal cirrhosis, in which shock and acute nephrosis occur frequently. In toxic hepatitis all three lesions, biliary, acute, and necrotizing nephrosis, are found together, with the first usually in the background [2336, 2480]. In biliary obstruction, biliary nephrosis is usually very severe, and features of acute nephrosis may also be present [127], especially following decompression surgery on the biliary tract [1998]. Acute nephrosis may follow biliary decompression and other operations even without preceding jaundice.

Renal Function in Liver Disease

Alteration of renal function has been reported in various hepatic diseases. Albuminuria and cylinduria are frequent findings in almost all liver diseases. In studies on human volunteers inoculated with infectious hepatitis, a decreased concentrating power, albuminuria, hematuria, excretion of casts (often bile-pigmented), and slightly reduced urea clearance have been demonstrated [982]. In fulminant viral hepatitis, the level of blood non-protein nitrogen is usually not elevated [2085]; it is elevated in delayed hepatic necrosis [2083]. In severe, nonfatal, toxic hepatitis, azotemia, especially elevation of blood-urea level, is frequent [2277, 3113], although glomerular filtration is not reduced [2639]. The creatinine level increases but not necessarily to the same degree as that of urea

or NPN. In cirrhosis, renal function is disturbed, especially during accumulation of ascitic fluid. The glomerular filtration rate and renal plasma flow are reduced [981, 1959, 2533, 2639]. However, the sodium retention characteristic of this phase may develop without these alterations of renal function [946]. Nocturnal diuresis is also present in cirrhosis with ascites [1657]. In severe cirrhosis, blood urea is frequently increased [2277]. Azotemia occurs in extrahepatic obstruction, especially in severe liver damage [2277], with a reduction of urea clearance and of the glomerular filtration rate [2639]. Release of biliary obstruction sometimes causes renal failure, which may be fatal [362] (see Hepatorenal Syndrome, later in this chapter).

CAUSE OF AZOTEMIA IN HEPATIC DISEASE. Azotemia results chiefly from elevated levels of urea, amino acids, and uric acid. An unknown peptide fraction also increases. The uric acid increase is quantitatively the least important and is explained by faulty hepatic oxidative metabolism. The rise in amino acids is caused by faulty deamination by the damaged liver. The bulk of the nonprotein nitrogen is urea, which shows an erratic behavior. The elevated blood-urea level is the result of two antagonistic factors:

1. In liver damage, deamination is reduced, tending to decrease the urea level, as seen in some instances of acute hepatic failure in man.

2. In the majority of cases, the blood-urea level is elevated because of abnormal reabsorption of urea through damaged tubules.

The increased blood urea seen in milder conditions without oliguria has been called "prerenal azotemia in liver disease" but is actually a tubular or nonglomerular injury resulting in abnormal reabsorption of urea [2277]. This tubular injury possibly is a *forme fruste* of acute nephrosis and is caused by similar factors.

EXPERIMENTAL ANIMALS. Azotemia occurs in liver damage caused by allyl formate intoxication [2625]. Ligation of the common duct in rabbits does not increase the NPN except if hemoconcentration is produced [1578].

Correlation between Hepatic Failure and Renal Injury. The correlation between the degree of hepatic failure and renal injury on one hand and between the apparent renal injury and the functional impairment on the other appears to be very poor at first glance [1578]. However, if the morphologic entities are separated, the correlation becomes clearer.

HEPATORENAL SYNDROME. The term "hepatorenal syndrome" has been used to describe a variety of lesions including (1) parallel injury to both organs in toxic conditions (mainly described in the French literature); (2) the renal damage encountered in obvious hepatic diseases; (3) acute renal insufficiency with anuria following either trauma [2497] or surgery on the biliary tract, particularly decompression, as emphasized in the American surgical literature [362, 1476]. With the recent understanding of the renal injury associated with hepatic lesions, especially with the development of the concept of acute nephrosis, the term "hepatorenal syndrome" becomes obsolete. Moreover, some of the renal injuries in these conditions are only incidental shock lesions of little importance clinically as far as hepatic function is concerned.

Influence of the Kidney on the Liver

Experimental removal of the kidneys influences metabolic and enzymatic functions of the liver [2401]. Very high serum levels of vitamin A in the nephrotic syndrome are considered evidence of altered hepatic metabolism [1673]. The occurrence of hepatic cirrhosis in Fanconi's syndrome supposedly is caused by endogenous malnutrition resulting from the loss of amino acids in the urine [3242]. Little evidence of hepatic damage has been found in earlier stages of renal diseases. In the nephrotic stage of glomerulonephritis, few functional changes indicating hepatic injury have been found [364], and plasma esterase formation by the liver is not altered despite the hypoproteinemia [1885]. Central necrosis occurs only in fatal uremia [3470]. It is probably a reflection of both circulatory disturbances and retention of toxic substances. These changes are found most frequently in acute nephrosis.

From a clinical standpoint, the effect of renal injury upon phenomena usually associated with hepatic injury is more important than the effect upon hepatic structure. For instance, decreased renal function, especially if caused by passive congestion, aggravates ascites formation in cirrhosis largely because of impaired sodium excretion [2326]. Hippuric acid excretion is reduced in renal failure, thereby influencing the results of the test [1831].

Influence on Bile Pigment Excretion. In liver disease with renal failure urobilinogen and urobilins may be absent from the urine. The clearance of bilirubin is reduced in the presence of

renal damage [2775], and in chronic glomerulonephritis bilirubin may disappear from the urine even if the prompt-reacting level is high. Impaired renal function explains why the bilirubin level is much higher in parenchymal hepatic diseases, especially those of a toxic nature, than in biliary obstruction not associated with severe renal damage. The increase in serum bilirubin is also an index of renal function, and a sudden rise of a previously stable bilirubin level is an alarm signal indicative of deterioration of renal and hepatic function, especially in biliary obstruction [2500]. In dogs, the renal clearance of bilirubin is greater than in man, and consequently, after ligation of the common duct, the serum-bilirubin level never rises so high and cholemia develops much later.

RELATION BETWEEN THE LIVER AND THE CARDIOVASCULAR SYSTEM

Effect of the Liver on the Cardiovascular System. The effects of the liver are either humoral or mechanical. The humoral mechanisms are (1) the VDM-VEM mechanism (see Vasoregulatory Functions, earlier in this chapter, under Hepatorenal Relationships); (2) lack of neutralization of anti-diuretic hormones (see Posterior Pituitary Hormones, under Pituitary Gland, Chap. 62); (3) the provision of substrates for the renal pressure substances such as renin, since renin is ineffective in the hepatectomized dogs [845]. In cirrhosis the incidence of arterial hypertension is lower than expected [3290], although the liver plays a role in the cause and perpetuation of hypertension [243]. Similarly, atherosclerosis seems to be less common in liver disease, although the macromolecular lipoproteins are not changed [2594].

THE LIVER AS A FLUID DEPOT. The effect of the liver on the general circulation is especially manifest in cardiac failure, in which the liver contains large amounts of blood, thus relieving the lungs of some of the congestion. The liver serves not only as a depot of whole blood but also as a depot of tissue fluid stored in its interstitial spaces. The mechanism by which the liver retains blood is either mere dilatation of the hepatic veins and the sinusoids, with a gradient from the central vein toward the lobule, or active retention by contraction of hepatic vein sphincters (see Hepatic Vein Sphincters, under Regulation of Hepatic Blood Flow, Chap. 18). An additional factor influencing fluid storage in the liver is the diaphragm, which compresses the spongelike organ.

BRADYCARDIA. Bradycardia in the presence of jaundice is usually considered a toxic effect of bile acids, although other retained biliary substances or nonspecific factors such as electrolyte imbalance play a role in hepatic insufficiency.

ELECTROCARDIOGRAM AND CARDIAC OUTPUT IN LIVER DISEASE. In viral hepatitis, a slow heart rate is found in 25 per cent of the cases [875], but no other significant alterations in the electrocardiogram, cardiac output, or arterial blood pressure were observed either at rest or after exercise [4]. In severe hepatic failure, cardiac output is increased. In one-third of the cases of cirrhosis, the resting cardiac output is increased, with a shortened circulation time, an increased stroke volume, a normal blood pressure, lowered peripheral resistance, and a decreased arteriovenous oxygen difference [1849]. Venous oxygen saturation is increased, possibly as a result of increased arteriovenous shunting. The electrocardiogram in cirrhosis shows prolongation of the QT interval [2491]. These findings are similar to those in beriberi, where they are caused by peripheral arteriolar dilatation. Gallbladder disease reflexly causes anginal pain and electrocardiographic changes [1513].

Effect of Circulatory Changes on the Liver. The liver is extremely sensitive to variations in the circulation insofar as changes in volume, rate of flow, and oxygen saturation are concerned. The effects of all these factors have been discussed under the results of ligations of individual vessels (see under names of individual vessels, Chap. 18), shock (see Hepatic Necrosis from Shock, Chap. 49), congestion (see Human Hepatic Injury from Congestion, Chap. 48), and anoxia (see main headings in Chap. 49). In various disorders of the heart without apparent cardiac failure, abnormal results are obtained in the hepatic tests, especially in tests of cephalin flocculation [1782] and Bromsulphalein clearance [2640].

RELATION BETWEEN THE LIVER AND THE SKELETAL SYSTEM

In long-standing extrahepatic biliary obstruction, especially in children with congenital biliary stenosis, bone changes develop which have been called "hepatic rickets" [3315]. Fat-soluble vitamin D is poorly absorbed in the absence of bile salts, and a specific effect of the liver on the utilization of vitamin D has been postulated [1459]. In addition, calcium absorption is reduced because of the associated steatorrhea. In adults, prolonged

extrahepatic biliary obstruction leads to osteomalacia.

Arthritis. A beneficial influence of jaundice and liver disease on rheumatoid arthritis has been claimed [1454]. On the other hand, abnormal results of hepatic tests have been found in rheumatoid arthritis [506, 1463]. In liver biopsy specimens, no significant structural alterations are found [2366]. The findings in necropsy specimens are nonspecific [134]. Abnormal results of hepatic tests are in part related to the hypergammaglobulinemia and hypoalbuminemia frequently found in rheumatoid arthritis. Similarly, in nonspecific arthritis, the flocculation tests yield abnormal results when the serum-gamma globulin level is elevated [1193]. Hepatic function and structure are not abnormal in gouty arthritis [3647].

RELATION BETWEEN THE LIVER AND THE CENTRAL NERVOUS SYSTEM

The relation between the liver and the brain has long attracted interest, but few concrete facts are available. The midbrain, particularly the hypothalamus, exerts a great influence on metabolism in general [3711] and probably on hepatic function, although these relations are as yet not well established. The changes in Wilson's disease (hepatolenticular degeneration) have been described (see Hepatolenticular Degeneration—Wilson's Disease, Chap. 53).

Influence of the Liver on the Brain

Brains perfused with simplified solutions maintain respiration and glucose uptake much longer if the perfusion fluid passes through the liver. This has been considered evidence of the ability of the liver to produce a factor necessary for cerebral metabolism [1141].

Neuropsychiatric Complications of Liver Disease. Nervous system complications, such as meningismus, encephalitis, neuritis, polyneuritis [1296, 1383, 3334], and tremor [217], occur in viral hepatitis. Neuritis of the Guillain-Barré type has been observed [3720], and occasionally psychotic reactions occur [1942]. Neurologic manifestations and hepatic coma associated with hepatocellular failure have been discussed previously (see Hepatic Coma, Chap. 23).

Morphologic Brain Changes in Hepatic Injury. In various experimental intoxications, such as carbon tetrachloride or phosphorus poisoning, associ-

ated with severe hepatic injury, alterations of the brain have been reported [1776], and in various human hepatic injuries edema, circumscribed demyelination, loss of Nissl granules, satellitosis with glial proliferation, and even hemorrhage have been seen [2917, 2918]. These changes must be differentiated from the cerebral effects of vitamin B deficiency, such as Wernicke's polioencephalopathy, which occur in nutritional liver disease but are not related to its severity.

Kernicterus. When bilirubin is present in the spinal fluid in adults (see Spinal Fluid Bilirubin, under Relation between Serum and Tissue Bilirubin, Chap. 21), bile pigmentation develops in circumscribed portions of the tuber cinereum, choroid plexus, pituitary gland, and dura, in addition to areas of encephalomalacia. On the other hand, in neonatal jaundice, such pigmentation occurs in the basal ganglia and in Ammon's horn cells and rarely in the cortex. This condition in neonatal jaundice is called "kernicterus" or "cerebral jaundice" [730]. It develops only in the first postnatal week, and the pigmentation is noted after the third day of life. Kernicterus is a serious condition, since it is associated with damage to the ganglion cells. It is the gravest complication of neonatal jaundice (see Hemolytic Disease of the Newborn, Chap. 49). Death may result within the first week, or permanent, crippling brain injury may develop in survivors, causing cerebral palsy or mental retardation.

DISEASES ASSOCIATED WITH KERNICTERUS. Kernicterus develops in jaundice without interference of bile flow and is associated with significant elevations of the indirect-reacting bilirubin. It is usually a complication of hemolytic disease of the newborn or erythroblastosis fetalis [3734], but it also occurs in familial nonhemolytic jaundice [688] and in jaundice developing in prematurity and severe infections [3734]. Extrahepatic biliary obstruction does not produce kernicterus.

CLINICAL FEATURES. Involvement of many of the brain stem nuclei (hence the name "kernicterus" or "nuclear jaundice") causes restlessness, eye signs, stiff neck, opisthotonos, and a high-pitched cry. Fever and convulsions are noted, and death occurs in about two-thirds of untreated children within the first week, although it may occur as early as the first day of life. The babies are not jaundiced at birth, but jaundice may develop in the first hours of life.

PATHOGENESIS. The pathogenesis of kernicterus is not established. Three factors are usually cited

1. Damage of the brain tissue by anoxia and anemia, with subsequent pigmentation of the damaged areas.

2. Toxic effect of the elevated level of indirect-reacting bilirubin [42, 749, 750, 3501], which has been explained as a competition for proteins with cytochrome. A serum-bilirubin level of 20 mg per 100 ml is considered critical [42]. In experimental animals, much higher levels are required [749]. The inability of the immature liver in the first days of life to excrete bilirubin contributes to the bilirubin elevation and thus to the kernicterus. Kernicterus has been observed with much lower bilirubin levels, which indicates that bilirubin elevation is not necessarily the main factor.

3. Abnormal permeability of the blood-brain barrier in the first days of postnatal life [3430] probably permits the abnormal bile imbibition.

The immaturity of the liver and the increased permeability account for the age predilection of the condition. Probably all three factors are present in varying degree in every instance. The importance of the bilirubin elevation is indicated by the excellent results of single or repeated exchange transfusions [42, 3502], which prevent the development of kernicterus. Exchange transfusions are

necessary if the bilirubin level approaches 20 mg per 100 ml.

Influence of the Brain on the Liver

Alterations of the liver in the presence of cerebral abnormalities have been demonstrated histologically [1735], chiefly by abnormal results of various hepatic tests [29]. In anxiety states, hyperexcretion of hippuric acid has been reported [2568]. In schizophrenia and in toxic psychosis, disturbances in hippuric acid synthesis have been found by some investigators [1057, 1148] but not by others [3650]. No characteristic changes were found in the results of the thymol turbidity or cephalin flocculation tests [2485]. In extrapyramidal diseases, abnormalities in results of some of the flocculation tests have been seen [1445]. Spinal cord transection has been claimed to prevent the effects of hypoxemia [1735]. Following spinal cord injury, hepatomegaly occurs [3324], and temporary Bromsulphalein retention develops [652]. In multiple sclerosis, hepatic function is not regularly altered [803]. Fatty liver develops as a result of mesencephalic damage and of increased intracranial pressure. This may occur because of pituitary changes.

TRAUMATIC HEPATIC INJURY

Traumatic injury to the liver is relatively frequent, and next to the brain, it is the organ most commonly affected by blunt violence. Since the injuries either quickly cause death from hemorrhage or do not necessarily produce significant functional or clinical alterations, they usually do not receive much emphasis. The surgical problem of hemostasis has attracted most interest. Traumatic injury to the gallbladder is not within the scope of this book. Surgical injury to the bile ducts, which more often involves the hepatic rather than the common duct [3620], has been discussed under strictures and surgical interruptions (see Strictures, also Accidental Surgical Interruption of the Common or Hepatic Duct, under Jaundice with Impairment of Bile Flow, Chap. 21).

Types of Trauma. Trauma to the liver results from penetrating or nonpenetrating, usually blunt, injuries. The majority of the former are bullet or stab wounds, with obvious implications. In penetrating thoracoabdominal wounds the liver is involved in 27 per cent of cases. Internal, blunt, or nonpenetrating injuries are largely the result of automobile injuries, falls, and blows [3659]. In these accidents the liver can be lacerated by broken ribs, crushed by impact of the ribs, especially if the organ is pressed against the spine, or ruptured, with the development of transcapsular lacerations. Central and subcapsular lacerations occur owing to internal stress and contrecoup injury [2076]. Rupture of the liver is facilitated by diseases which increase capsular tension and the friability of the organ. These include hepatitis, abscesses, cysts, malaria, and fatty liver. Postprandial hyperemia also enhances rupture, so that

a minor injury may result in unexpectedly severe damage. Rupture of the liver occurs as a rare complication of labor, usually associated with hepatic injury during pregnancy [3148]. Spontaneous rupture, as seen in the spleen in various diseases, does not occur in the liver.

MORTALITY. If minor injuries are disregarded, the mortality rate for lacerations or rupture of the liver is at least 10 to 25 per cent, regardless of the type of treatment [2168, 3143], and is often 60 per cent [2596, 3659]. Civilian wounds have a better prognosis than war wounds [3143]. During the first few days, death is caused by hemorrhage; after that, by septic or biliary peritonitis [2520]. Bleeding is usually prolonged and severe because (1) the liver is extremely vascular; (2) the walls of the hepatic veins are thin and have very little elastic tissue and no valves; (3) the diaphragm exerts a massaging effect; (4) the admixture of bile with blood delays clotting.

INTRAPARTUM RUPTURE OF THE LIVER. The liver ruptures in newborn infants as a result of birth injury. This uncommon complication was found 24 times in 2,000 autopsies on newborn babies [2664]. It occurs more frequently in large infants or in those with large livers, as in erythroblastosis fetalis or maternal diabetes [1304]. Asphyxia, with resulting hepatic congestion, seems to be a predisposing factor [1457]. The mechanisms for rupture in these infants are (1) increased pressure on the thorax, squeezing the liver out of the hollow of the diaphragm and putting undue tension on the hepatic ligaments; (2) direct pressure of the costal margin on the anterior surface of the liver; (3) direct trauma [1304].

Functional Changes. The effects of trauma on hepatic function are best known from animal experiments. In view of the great hepatic

Bromsulphalein retention, galactose tolerance, prothrombin time, and serum-alkaline phosphatase activity are not appreciably affected by massive trauma even when 50 per cent of the liver is damaged [2226]. Jaundice as an immediate sequela of the injury is rare, except as a result of the infrequent rupture of the gallbladder [3102] or bile ducts [2236] with biliary peritonitis or infarction of the liver [3209, 3659]. Jaundice developing late is the result of traumatic cholangitis or liver abscess.

Complications. Various complications of trauma to the liver include subphrenic, intrahepatic, and subhepatic abscesses after infections of hematomas, cyst formation in the liver, biliary peritonitis, and traumatic fistulas [1059, 2348]. Interruption of the blood supply to part of the liver as a result of trauma usually leads to infarction [2348, 3659]. Isolated amputated pieces, as well as the area in the vicinity of the traumatized portion, show ischemic necrosis, which involves both parenchymal and mesenchymal structures and produces the picture of an anemic infarct. Fissures containing blood may appear in the surrounding, otherwise intact, parenchyma. Within a few days after the trauma, bile duct regeneration, starting from the portal tracts, and active fibroplasia produce a zone of demarcation. Finally, around extensive trauma, a zone is noted which appears to be cirrhotic. Smaller injuries heal, almost without scar formation.

EFFECT OF NECROTIC LIVER TISSUE. Traumatized or necrotic hepatic tissue within the liver or peritoneal cavity is said to exert a specific toxic effect, more toxic than that of any other organ. These changes, however, are more likely the result of either biliary peritonitis or secondary infection. Relatively large pieces in the peritoneal cavity have been well tolerated in man, and secondary attachment of completely detached fragments has been reported [2348]. This supports the finding that human liver is bacteria-free. Secondary infections of necrotic areas are common. The so-called hepatorenal syndrome following hepatic trauma [110] is a nonspecific acute nephrosis caused by shock (see Hepatic Necrosis from Shock, Chap. 49).

FOREIGN BODIES IN THE BILIARY PASSAGES. Rare and usually late complications of trauma or surgery are foreign bodies in the biliary ducts [1440, 333]. Gauze, pieces of instruments, or fragments of rubber tubing have been found in the common hepatic ducts many years after gallbladder sur-

gery. Similarly, bullets or shell fragments have migrated through the liver over a period of years, becoming lodged in the extrahepatic bile ducts and causing obstruction.

Medicolegal Aspects. The relation between trauma and liver diseases is a challenging problem of medicolegal interest. A traumatic etiology can be considered in the development of abscesses, cysts, arterial aneurysms, and portal or hepatic vein thrombosis. On the other hand, preceding trauma has been claimed to be a causative factor of diseases with poorly established etiology, such as hepatitis or cirrhosis [3209]. The effect of shock resulting from trauma produces central necrosis owing to circulatory hepatic injury, but this quickly subsides with recovery. Serum hepatitis may also be considered an indirect but readily apparent sequela of hepatic trauma. Cases have been reported in which acute and even fatal hepatitis resembling acute yellow atrophy follow trauma after varying intervals without the possibility of viral hepatitis. Courts have acknowledged the relationship [3209]. The relative rarity of such occurrences, especially in recent years with improved observation, makes it very doubtful that such relations are more than coincidental.

SEVERE TRAUMA NOT INVOLVING THE LIVER

Severe trauma not involving the liver may cause structural hepatic alterations and changes in the results of hepatic tests, mainly owing to the associated shock. Toxic tissue-breakdown products, especially in burns, have been accused of playing an additional role. Since preservation of blood-sugar levels takes preference over other functions, excretion of dyes and bile pigment may be impaired, while hypoglycemia does not occur.

HYPOTHERMIA

Exposure of normal animals to cold causes rapid disappearance of hepatic glycogen [433]. This disappearance is prevented by thyroidectomy [2307]. In man, hypothermia with inanition does not lead to loss of liver glycogen, apparently because of concomitant changes in the thyroid gland [2307]. Animals reared in a cold, damp environment have enlarged livers, with irregular areas of congestion and ischemia. Cloudy swelling, vacuolization, fatty metamorphosis, and loss of basophilia occur early, and if the animals live, necrosis and fibrosis de-

velop irregularly throughout the liver [55, 2405].

On the other hand, hypothermia reduces the incidence of hepatic necrosis on protein- and vitamin E-deficient diets [2404], increases the survival rate after hepatic anoxia in dogs produced by clamping the hepatic artery [2691], and prevents fatty livers in rats on low-choline-high-fat diets [3001]. These phenomena have been explained on the basis of an alteration of body metabolism by which choline is spared, more efficiently utilized, or synthesized in larger amounts.

IONIZING RADIATION

The effect of irradiation on the liver depends on how it is administered, i.e., externally by x-rays, or internally by radioactive substances, on the size of the dose, and (if by radioactive substances) on their mode of radioactive decay and on their pathways of metabolism and disposal by the liver. In general, all observers agree, at least on the basis of morphologic criteria, that the liver is relatively insensitive to radiation injury [3583].

External Irradiation

Most of the available information on the effects of x-rays on the liver comes from studies employing total body radiation in small animals. This method yields almost the same results as irradiation directed to specific areas of the body; furthermore, it can easily be standardized with x-ray therapy machines. The irradiation is applied until the animal dies or, more commonly, is given in LD₅₀ doses as a single exposure. The effects of the irradiation on the liver have been explained partly as a nonspecific effect on cells in general and partly as a specific effect on hepatic structure and function.

Nonspecific Effect on Cells. Alteration of cell structure and function is the result of a direct action of the irradiation on macromolecular structures, causing depolymerization [3342]. Irradiation also causes ionization of intracellular water, with the formation of nascent oxygen, hydrogen peroxide, or other combinations of hydrogen and oxygen which are powerful oxidizing agents [422, 3342]. Apparently the most important effect is that on mitosis, especially in growing liver tissue or in livers regenerating after partial hepatectomy [422]. This is manifested in (1) reversible delay in cell division; (2) visible chromosome damage; (3) change in mitotic figures; (4) arrested mitosis with inhibition of cell division. Cell division in

regenerating liver is reduced 50 per cent by doses of total body radiation of 1 roentgen equivalent per hour for 48 hours. Irradiation of tissues also depresses formation of desoxypentose nucleic acid [1474]. Part of this effect is humoral in nature, because selective irradiation of the liver and spleen depresses DNA formation throughout the body and in transplanted tumors [1718]. Mitochondria of the hepatic cells are also altered by total body radiation [2104].

Specific Effects on Hepatic Structure or Composition. **STRUCTURAL CHANGES.** Supervoltage irradiation to the gastrointestinal tract in man may produce significant alterations of the liver, including fibrosis, necrosis of the left lobe, which is maximally exposed, and stricture of the common duct [396]. Total body irradiation to rats on deficient diets increases the incidence of cirrhosis formation [3583]. In rats on normal diets, it causes enlargement of the liver within 12 hours, and this usually persists for 4 days [2086]. This is not the result of an increased content of fat or solids but of hypertrophy of the individual parenchymal cells.

GLYCOGEN. Changes in liver glycogen depend on the amount of irradiation given. If hamsters are exposed to massive doses until they die, usually 110,000 roentgens in about 3.5 hours, or salamanders to 176,000 roentgens in 80 minutes, the liver glycogen level drops to very low amounts, histologically and chemically, the drop being greater in the hamster than in the salamander [1978]. In smaller but still lethal doses (880 roentgens) or given to fasted rats, glycogenolysis is retarded and after 24 hours normal amounts of glycogen are still found [769]. Adrenalectomy prior to irradiation increases the amount of glycogen remaining. Smaller doses of irradiation, up to 500 roentgens, raise the glycogen content of the liver [2834].

LIPIDS. The concentrations of hepatic fat [63, 2086], cholesterol, phospholipid, and vitamin [636] are not changed by irradiation. Previous reported fatty liver caused by x-rays [1925] was apparently the result of starvation.

ENZYMES. Enzyme changes are surprisingly undramatic. For instance, the activities of mercaptosulfhydryl enzymes drop following irradiation [17, 2086], together with an increase in hepatic sulfhydryl content [769], while those of nonmercaptosulfhydryl enzymes, such as esterase, arginase, rhodanase, succinoxidase, and lactic dehydrogenase, are unchanged [1017, 2086]. Alterations in cytochrome oxidase activity, if present, are slight [769, 2086].

Catalase level has been reported to drop [989], although this has been denied [2086]. Hepatic alkaline phosphatase activity drops slightly between the second and fourth day following irradiation. Some of the enzyme changes are not the direct result of irradiation but are mediated through the adrenal gland [3331].

OTHER CONSTITUENTS. The content of hepatic water, sodium, or potassium is unchanged, but that of inorganic phosphorus and of labile organic, or ATP, phosphorus drops during the first three days, returning to normal during the fourth or fifth day [769]. ATP phosphorus may continue to rise above normal. Iron is increased in the liver following sublethal irradiation for 2 weeks, returning to normal by the third week [2086]. This is exaggerated in rats that are made anemic prior to exposure.

Effects on Hepatic Function. Irradiation causes some changes in the results of hepatic tests that can not be correlated with structural changes. Serum-cholesterol level rises, with a drop in the ester fraction [666]. The A/G ratio decreases [666, 3435] because of a progressive drop in albumin [2384] and a rise in alpha globulin [1182, 3566]. Some of the drop in A/G ratio can be abolished by ether extraction and is probably caused by increased serum beta lipoproteins [2830, 3342, 3566]. Gamma globulin level also drops [3566]. Turnover of serum lipid and phospholipid is increased [942]. With nonfatal doses of irradiation, serum-alkaline phosphatase activity drops slightly, while larger doses cause a precipitous drop in fed or fasted animals [2086]. The results of some of the hepatic tests, such as tests of thymol turbidity and prothrombin time, are unchanged [666]. In man, large doses of x-rays or radium for tumor therapy have reportedly led to Bromsulphalein retention, abnormal cephalin flocculation, fluctuation of serum gamma globulin, and a decrease in serum lipids [3117]. Irradiation toxicity is increased by low-protein-high-fat diets, and this is not influenced by addition of cysteine or methionine to the diet [3103].

Internal Radiation

The uptake by the liver of administered radioelements has been studied in many species, usually as part of an investigation of the distribution of an isotope in the body. Elements studied include phosphorus, P^{32} [2820], gold, Au^{198} [3712], silver, Ag^{111} [1126], hafnium, Hf^{181} [1783], mercury, $Hg^{203,205}$ [2723], plutonium, Pu^{239} [2031, 2957],

and yttrium, Y^{91} [2957]. The amount taken up by the liver is determined by the physical properties of the material, especially the size of the particles in solution or colloidal suspension [2957, 3712]. Most of the particulate matter is picked up by the reticuloendothelial cells of the liver and spleen, regardless of the route of administration [1126]. Considerable species variation is found in the disposition of such material [3390]. Some substances, such as hafnium [1783] and thorium [2155], are retained in the liver for long periods of time, while others, such as colloidal silver [1126] or mercury in the form of diuretics [2723], are excreted in the bile. Certain chemical forms permit rapid incorporation of radioactive material in the hepatic parenchymal cells. Phosphorus in the form of phosphate is rapidly incorporated in many intracellular compounds. This has permitted the study of the toxic effects of internal irradiation.

Hepatic Injury from Internal Irradiation. Direct damage to the liver by radioactive material is difficult to produce. The earliest information came from the study of radium poisoning in watch-dial painters and the few animal experiments with radium which followed. Production of large amounts of radioactive isotopes has enabled the study of the radiotoxicity of material largely incorporated in the hepatic cell, such as P^{32} , and of material largely taken up by the reticuloendothelial system, such as radioactive colloidal gold.

RADIUM. Ingested or injected radium accumulates, mainly in the liver, at first and then gradually disappears [2086], with some always remaining. Acutely, administration of radium to dogs causes hemorrhagic necrosis of the liver, while implantations of radium lead to formation of hepatocellular tumors after 12 months. Thorium in the form of Thorotrast, which was formerly used diagnostically for roentgenologic visualization of the spleen and liver, leads to sarcoma in the liver in man [2155]. Plutonium injected into animals produces necrosis with jaundice and ascites and scars in the livers of survivors [2031].

RADIOACTIVE GOLD. Radioactive gold, injected intraperitoneally for control of ascites caused by peritoneal carcinoma implants, accumulates in reticuloendothelial cells of the liver. However, long-term effects are not seen after single doses, since the half-life of Au^{198} of 2.7 days is so short in contrast to the many years for the natural radioelements, radium and thorium. Fatal doses of radioactive gold in guinea pigs cause focal hepatic necrosis with radioactivity in the necrotic areas

demonstrable by autoradiography [1020]. The maximal changes occur after 2 weeks, and at that time myeloid metaplasia in the liver complicates the histologic picture. Radioactive gold produces intense regeneration, with hepatocellular giant-cell formation in addition to necrosis [1834]. Eventually postnecrotic cirrhosis develops. On repeated injection to young dogs, cirrhosis with centrilobular fibrosis and ascites develops. In mice prolonged administration of radioactive colloidal gold leads to bizarre regeneration, cirrhosis, and formation of hepatic tumors [3395].

RADIOACTIVE PHOSPHORUS. Radioactive phosphorus accumulates to the greatest extent in the liver initially. It causes no hepatic changes even when given in lethal doses intraperitoneally [1833] or subcutaneously [1238]. Preceding fatty liver from choline deficiency and carbon tetrachloride intoxication or choline supplementation have no effect on toxicity or on hepatic changes [668]. High-protein diets increase the toxicity [668], although decreased toxicity in protein-depleted rats has also been reported [1640].

HYPERSENSITIVITY

The evidence for a relation between the liver and altered reactivity of the body is far from convincing.

EFFECT OF LIVER UPON ALLERGY. An effect of the liver upon allergy is suggested by various observations. Sulfonamide idiosyncrasy more readily develops in the presence of liver damage [1889]. Urticaria and dermatitis occur in liver disease, and eosinophilia is often noted [3223]. Since sensitization, as well as reaction, can occur via the gastrointestinal route, interposition of the liver in the portal circulation should influence this by removal or "detoxification" of the allergens. An example of such a phenomenon is the hemoclastic crisis, or leukopenia and hyperfibrinogenemia, which occurs more readily in liver damage after protein feeding [943]. On the other hand, blockade of the reticulo-endothelial system suppresses anaphylactic shock.

ALLERGIC REACTIONS IN THE LIVER. Some observations suggest that the liver and gallbladder are target organs for hypersensitivity reactions. The available references present a confusing picture

[1735]. In anaphylactic shock, the NPN of the liver increases. Nonspecific reactive hepatitis has been reported in experimental hyperergic conditions [1402].

Experimental Allergy. Lesions have been found in animals made hyperimmune during antiserum production, for instance against diphtheria and tetanus, in anaphylactic shock, and in studies in which attempts have been made to produce anti-liver antibodies [1735]. The pathogenesis of the changes is uncertain. A direct effect upon the hepatic cells is possible, as well as a primary disturbance of the hepatic circulation. For instance, in dogs spasm of the hepatic vein with increased hepatic lymph flow has been demonstrated in anaphylactic shock (see Hepatic Necrosis from Shock, Chap. 49). Reference has been made to the vascular changes in histamine shock, although a primary effect upon the liver can not be excluded [945].

Hepatic Reactions in Human Allergies. Severe allergic reactions, such as angioneurotic edema or serum sickness, are sometimes associated with hepatic disease and jaundice. Hepatic disorders in light-sensitive patients have been recorded [3396]. Hepatic vascular involvement is found in hypersensitivity angiitis [3698]. Toxic hepatitis as a result of idiosyncrasy (see Idiosyncrasy, under Exogenous Factors, Chap. 41) and allergic cholangiolitis have been described (see Allergic Cholangiolitis, Chap. 41), as has allergic involvement in the biliary tract [3396]. In allergic tissue eosinophilia, such as Loeffler's syndrome and various parasitic diseases, periportal infiltration with eosinophils is noted, which disappears in biopsy specimens after treatment with steroid hormone or corticotropin [781].

ALLERGIC REACTIONS IN VIRAL HEPATITIS. In man the relation between hypersensitivity reactions and viral hepatitis is puzzling. Some of the initial symptoms of this disease, such as joint pain and dermatitis, may be allergic manifestations, and the mesenchymal reaction in the liver may also be allergic in nature [1376, 2409]. Moreover, allergic reactions and colds possibly predispose to the development of viral hepatitis [2308]. Antibody formation in the liver in hepatitis [498, 2221] has been discussed before (see Antibody Formation, Chap. 14).

PART VIII

Appendix

PRINCIPLES OF DIAGNOSIS OF LIVER DISEASE BASED ON COORDINATED USE OF FUNCTIONAL AND STRUCTURAL OBSERVATIONS

The clinical application of the hepatic tests has been argued more extensively than the use of function tests for other organs, and the resulting confusion has often deterred proper usage. The hepatic tests differ from the other function tests in several respects. The renal or pancreatic function tests, for example, directly measure the basic functions of that organ, while only few hepatic tests do so for the liver. Most of the hepatic tests in wide use provide only circumstantial evidence of altered activity within the hepatobiliary system. They usually demonstrate complex alterations such as hepatic-cell degeneration, cholestasis, and inflammation. When dealing mainly with pathologic phenomena, the histologic demonstration of such entities by liver biopsy becomes a valuable supplement and the histologic observation deserves correlation with biochemical studies. In clinical practice, the hepatic tests are superior to liver biopsy in some instances, while in others the opposite is true [2242, 2243].

The hepatic tests are used more often for purposes of clinical diagnosis than tests measuring function of other organs. However, the laboratory diagnosis of hepatobiliary diseases should remain ancillary to clinical observation. Nevertheless, if properly applied, the results of the tests can become crucial in certain situations.

Use of Multiple Tests. Several hepatic tests are performed in each patient for the following reasons:

1. The clinical entities of hepatobiliary disease are usually composed of several functional and structural pathologic features, which are not all present to the same degree. Therefore, tests indicating different features are employed.

2. Most structural abnormalities are associated

with alteration of several functions. For instance, in hepatic-cell degeneration, protein synthesis, glycogen storage, and pigment metabolism are deranged to varying degrees. Therefore, several tests indicating different aspects of the same feature should be used.

3. Unexplained abnormal results are found in the absence of hepatobiliary disease with almost every hepatic test. If such results are not caused by laboratory errors, they are designated "biologically false positive" reactions [2640]. Similarly, almost no test yields abnormal results in all patients with even severe hepatic insufficiency established clinically or by other laboratory procedures; therefore "biologically false negative" reactions are also encountered.

False Positive Results. IN NORMAL PERSONS. Review of the tables presented for each hepatic test indicates that in entirely normal subjects, such as students, nurses, or doctors, abnormal results are found in about 2.5 per cent. With some tests, such as the colloidal gold test or cholesterol ester ratio, abnormal results are reported in up to 5 per cent of normal persons.

IN HOSPITAL CONTROLS. In approximately 10 per cent of hospitalized patients suffering from conditions without any influence upon the liver, e.g., hernias and fractures, abnormal results in the hepatic tests are found. This is possibly explained by the presence of a clinically inconspicuous anicteric viral hepatitis.

IN PATIENTS WITH NONHEPATIC DISEASES. In patients with internal diseases in whom involvement of the liver can not be entirely excluded, the percentage of abnormal results becomes relatively high. The cholesterol/ester ratio is abnormal in one-fifth of such cases; the serum alkaline phos-

phatase and the thymol turbidity in one-third of the cases; and cephalin flocculation and galactose tolerance in about one-half of the cases. Bromsulphalein retention, colloidal red flocculation, and hippuric acid excretion are even more often abnormal. The following conditions influence liver function and thus lead to temporarily abnormal results: infectious diseases of all types (especially pneumonia, malaria, lymphopathia venereum, tuberculosis), rheumatoid arthritis, rheumatic fever, shock, hyperthyroidism, gastrointestinal diseases, renal diseases, alcoholism, and nonspecific febrile reactions [1482, 2640]. Abnormal results can be explained by serum-protein changes, which are seen particularly in infectious or renal diseases, or by a clinically inconspicuous toxic hepatitis. Abnormal results in this group are therefore not unexpected and usually do not present a differential diagnostic problem.

Significance of Biologically False Reactions. If the number of abnormal results in control groups is combined with the relatively few instances in which such results present a differential diagnostic problem in patients with nonhepatic diseases, biologically false positive results with each test are expected in about 10 per cent of patients without clinically significant liver disease. False negative results also occur in approximately 10 per cent of instances with almost every hepatic test. The error caused by the biologically false positive and negative reactions is best counteracted by performance of several hepatic tests, preferably with different physiologic bases [2640]. Biologically false positive, as well as false negative, reactions are especially common with the tests for hepatocellular degeneration.

Occasionally histologic findings represent a false positive or negative result. In the presence of various infectious diseases or in intoxications without primary hepatic involvement, changes of hepatic parenchyma and mesenchyma in the form of nonspecific reactive hepatitis are encountered which are rather dramatic (see Nonspecific Reactive Hepatitis, Chap. 41). Histologic differentiation from a primary hepatic disease is often impossible. Similarly, in some forms of cirrhosis or in pneumonia, the liver appears rather normal on biopsy. In the presence of significant functional derangements.

Physiologic Variations in Results of Hepatic Tests. Physiologic variations determine the outcome of the hepatic tests. The preceding diet influences galactose tolerance, because hepatic glyco-

gen and fat stores determine glycogen formation and deposition. Similarly, hippuric acid formation depends upon available glycine and therefore on dietary factors [173]. Intravenous administration of glucose may restore abnormal hippuric acid synthesis [3112].

MENSTRUATION AND PREGNANCY. Menstruation influences hepatic function and therefore the results of some tests. For instance, on the first day of the menstrual period, hippuric acid synthesis is depressed [1446]. During normal pregnancy in animals, pentose and desoxypentose nucleic acid turnover [477, 2727] and the cytoplasmic basophilia on the periphery of the lobule [477] are increased; they return to normal during lactation. In man, changes in hepatic function have been described, particularly in the last trimester, usually near term [1664]. Hippuric acid synthesis is abnormal until about the seventh or eighth post-partum day [2512]. Bromsulphalein retention and thymol turbidity are slightly increased, along with an increase in alpha and beta globulins, while gamma globulin is decreased [2058]. Normal pregnancy has no effect on the serum-protein changes in liver disease [2130]. Cephalin flocculation is abnormal in about 30 per cent of pregnant women [748, 2873, 3451], possibly as an indication of toxemia in some instances [2590] (see Hepatic Necrosis in Eclampsia, Chap. 49). Increased serum-bilirubin levels and urinary bilirubin excretion with decreased bilirubin tolerance have been described [1664, 3130]. Minor chemical [477, 1043] and morphologic [1588, 2448] changes in the liver and gallbladder are seen in normal pregnancies.

AGE. In persons over sixty years of age, abnormal results in hepatic tests are fairly common without demonstrable evidence of hepatic disease. In 97 per cent of older people, the results of at least one test were abnormal [2693]. In the order of decreasing incidence, abnormal results are found in cholesterol/ester ratio, cephalin flocculation, thymol turbidity, Bromsulphalein retention, and hippuric acid synthesis [2693]. The impairment of the hippuric acid synthesis has been considered to be caused by a defect in the glycine supply [3208]. Such alterations also include changes of the electrophoretic protein partition [2694] and increases in serum cholesterol [3154].

Selection of Tests. The selection of the hepatic tests from the many available is a challenge in their clinical use. This challenge has been met by using systems of several hepatic tests, the one best known being the liver profile of Watson and his

Table 63 Relation between Certain Morphologic Alterations in Hepatobiliary Diseases and the Results of the Commonly Used Hepatic Tests

Alteration	Gamma globulin	Zinc sulfate turbidity	Cephalin flocculation	Thymol turbidity	Serum albumin	Serum esterase	Serum alkaline phosphatase	Serum cholesterol	Cholesterol/ester ratio	Bromsulphalein	Urinary urobilinogen
Hepatic-cell degeneration	0	±	++	++	++	++	±	↓	↓	++	++
Inflammation.....	++	++	±	±	0	0	0	0	0	0	0
Fatty metamorphosis....	0	0	0	0	0	0	0	0	0	++	0
Cholestasis.....	0	↓	0	0	0	0	+++	↑	↓	+	↓
Cirrhosis.....	+++	+++	+	+	0	0	0	0	0	++	0

Table 64 Frequency of Occurrence of Certain Morphologic Alterations in Various Hepatobiliary Diseases

Alteration	Viral hepatitis	Toxic hepatitis	Uncomplicated biliary obstruction	Infected biliary obstruction	Protracted biliary obstruction	Cirrhosis
Hepatic-cell degeneration.....	++-+++	++	0	++	++	0-+++
Inflammation.....	++	+-++	0	++	++	+-+++
Fatty metamorphosis..	0	+	0	0	0	0-+++
Cholestasis.....	±	+	+++	+++	+++	±
Cirrhosis.....	0	0	0	0	+	++-+++

coworkers [1519, 3510]. Another system is the "hepatic star" arranged graphically to take into account the different processes which, in various combinations, produce hepatobiliary diseases [1270]. Many other combinations of tests have been described [415, 1254, 1521, 1606, 2240, 2641, 2815, 2973, 3136, 3310]. The relation of the results of tests to the histologic features is summarized in Tables 63 and 64 as a basis for clinical application. In the following discussion, the attempt is made to select tests for specific purposes.

Sensitivity of Tests. Several factors obscure the sensitivity of the hepatic tests, especially the large hepatic reserve and the regenerative ability of the liver [1606]. None of the tests indicates damage when less than 80 per cent of the liver is destroyed in the experimental animal [339]. Moreover, the sensitivity depends on the stage of the disease. Very sensitive tests become nonspecific, because minor alterations of hepatic function alter the results. Divergent claims have been published as to the comparative sensitivity of the different tests. For instance, cephalin flocculation, Bromsulphalein retention, intravenous hippuric acid tolerance, and thymol turbidity at a less alkaline pH were

considered the most sensitive; oral hippuric acid tolerance, urinary urobilinogen excretion, and the thymol turbidity at more alkaline pH were thought to be of intermediate sensitivity; while prothrombin-time response after vitamin K and serum-cholesterol level were apparently of low

Table 65 Sensitivity of Various Hepatic Tests

- 1. Most sensitive
 - a. Bilirubinuria
 - b. Prompt-reacting serum bilirubin
 - c. Urobilinogenuria
 - d. Bromsulphalein
 - e. Thymol turbidity (not in cirrhosis)
 - f. Serum alkaline phosphatase
- 2. Moderately sensitive
 - a. Cephalin flocculation
 - b. Zinc sulfate turbidity
 - c. Cholesterol/ester ratio
 - d. Serum esterase
 - e. Intravenous hippuric acid tolerance
 - f. Galactose clearance
 - g. Prothrombin response to vitamin K
 - h. Serum albumin
- 3. Nonsensitive
 - a. Aminoaciduria
 - b. Low total cholesterol
 - c. Hypoglycemia

sensitivity [2242, 2243]. In animals, Bromsulphalein and rose bengal clearance and serum-alkaline phosphatase activity were felt to be of comparable sensitivity [838]. Sensitive tests are helpful for screening purposes and for the establishment of toxic effects of drugs. The moderately sensitive tests, reflecting definite but not severe liver damage, assist in the differential diagnosis of jaundice and the management of hepatic disease. The tests in the nonsensitive group are used as alarm signals and for prognostic purposes (Table 65).

APPLICATION OF HEPATIC TESTS AND LIVER BIOPSY AS ANCILLARY PROCEDURES IN CLINICAL PROBLEMS

Differential Diagnosis of Jaundice

This differential diagnosis is the most important practical application of the hepatic tests. It is in effect a "therapeutic differential diagnosis" [1606], to separate "surgical" jaundice (from tumors, stones, or strictures) from "medical" jaundice (from hepatitis or cirrhosis). Hemolytic jaundice is not considered here, since it can be relatively easily established by other tests.

Basic Principles. The basis of the differentiation is the finding of abnormal results of tests indicative of hepatic-cell degeneration and of normal results of tests indicative of cholestasis in medical jaundice and vice versa in surgical jaundice. Intrahepatic tumor metastases or lymphomas producing jaundice belong to the medical group, since the jaundice results from intrahepatic processes not amenable to surgery. The following findings are recommended for appreciation of hepatic-cell degeneration:

1. Abnormal cephalin flocculation
2. Increased thymol turbidity
3. Urobilinogenuria
4. Low serum-cholinesterase level
5. Decreased cholesterol/ester ratio

In view of the frequent occurrence of biologically false positive tests, abnormal results in at least two of these tests are required. Abnormal results of both thymol turbidity and cephalin flocculation should be supplemented by abnormal findings in one of the other tests. The following tests are used for the recognition of cholestasis:

1. Absent urinary urobilinogen
2. Serum-alkaline phosphatase activity above 15 Bodansky units
3. Hypercholesteremia

Any one of these findings suffices in the presence of jaundice.

Exceptions to the Basic Principles. SECONDARY HEPATIC-CELL DEGENERATION IN SURGICAL JAUNDICE. This is caused by prolonged extrahepatic biliary obstruction (see Biliary Hepatitis, Chap. 47) or by secondary bacterial infections of the portal tracts (see Infected Biliary Hepatitis, Chap. 47). Laboratory evidence of both hepatic-cell degeneration and cholestasis is found in this group of patients.

INCOMPLETE OR INTERMITTENT EXTRAHEPATIC CHOLESTASIS. This results in fluctuating findings indicative of cholestasis in cases of surgical jaundice. When no cholestasis but slight liver damage is found at the time of examination, medical jaundice is probably present.

INTRAHEPATIC CHOLESTASIS, OR "CHOLANGIOLITIS." This produces abnormal results in tests indicating cholestasis in medical jaundice.

Outline for Laboratory Differential Diagnosis of Medical and Surgical Jaundice. Application of the findings presented permits the following scheme for the differential diagnosis between surgical and medical jaundice [2625]:

1. If hepatic-cell degeneration is present, with two or more tests showing abnormal results, and cholestasis is absent, the case is considered medical, with the following exceptions: (a) intermittent obstruction by a stone, recognized by fluctuating urinary urobilinogen excretion; (b) false positive results in a patient in whom an abdominal mass or a history of colic is the only clue to the diagnosis.

2. If evidence of hepatic-cell degeneration is absent and cholestasis is present, the case is considered surgical, the exception being intrahepatic cholestasis, or "cholangiolitis" (see Cholangiolitis and Pericholangiolitis, Chap. 46).

3. If both hepatic-cell degeneration and cholestasis are present, one should follow the results of the flocculation tests. If the results of these tests disagree, most significance is given to normal zinc sulfate turbidity, and next to abnormal cephalin flocculation and thymol turbidity. When the zinc sulfate turbidity is normal, the case is considered surgical, with hepatic-cell degeneration produced by prolonged biliary obstruction. The exceptions are the rare cases of toxic, mainly chemical, hepatitis in which Kupffer cell proliferation and gamma globulin elevation are absent and which can usually be suspected from the history. If cephalin flocculation or thymol turbidity in this group is

abnormal, the case is medical, with the following exceptions:

a. Secondary bacterial infection in extrahepatic cholestasis with excessive gamma globulin production, probably outside the liver. The condition is recognized as surgical jaundice by clinical signs of septicemia, such as chills, fever, and leukocytosis.

b. False positive results for hepatic-cell degeneration. Again an abdominal mass or a history of colic may lead to the correct diagnosis.

Roentgenologic visualization of the biliary system by newer dyes or by direct injection of dye into the gallbladder or extrahepatic bile ducts may provide a simple means of making this differential diagnosis.

Differential Diagnosis of Neonatal Jaundice

Jaundice in the neonatal period is very common and presents two problems of management. In the first days of life physiologic jaundice and other forms not causing kernicterus must be differentiated from hemolytic disease of the newborn, with its tendency to kernicterus, because exchange transfusions can prevent this complication. Later in infancy the differential diagnosis of surgical and medical jaundice becomes important, although only a few instances of surgical jaundice primarily caused by malformations can be corrected. The laboratory procedures used in differential diagnosis in adults are of limited value, because the hepatic cells and the reticuloendothelial system are immature and do not function well. Furthermore bilirubin appears in the urine in instances of jaundice without impairment of bile flow in infants, because hepatocellular damage is a frequent complication. The most reliable criteria seem to be the time of appearance and the height and duration of jaundice.

Jaundice which appears on the first day of life and rapidly increases to above 10 mg per 100 ml suggests hemolytic disease of the newborn. The diagnosis may be confirmed by a positive result of a Coombs test and a low hemoglobin in cord blood. This blood should be examined in all instances where Rh antibody titers have risen in mothers before parturition. Physiologic jaundice in premature infants may rarely have a course similar to that of hemolytic disease of the newborn, and the danger of kernicterus is present in these instances.

Jaundice appearing at the end of the first week or during the second week may be the result of

atresias, in which case it increases slowly but persistently to high levels, at which it becomes stationary. Jaundice at this time may also be caused by transplacental transmission of viral hepatitis or other infectious agents. In these instances the course of the jaundice is variable, and urobilinogen may be present in the urine. Umbilical infections also appear at this time and are indicated by septicemia. Operative cholangiography is of value in the differential diagnosis.

Differentiation between Extrahepatic and Intrahepatic Cholestasis

The separation of extrahepatic cholestasis, caused by tumors, calculi, and strictures and therefore surgical in nature, from intrahepatic cholestasis, caused by cholangiolitis and medical in nature, can seldom be made by clinical methods. A history of exposure to arsenicals or viral hepatitis speaks for intrahepatic cholestasis. A large or tender gallbladder or abdominal masses favor extrahepatic cholestasis. This differentiation can not be made reliably by biochemical methods. Elevation of serum-alkaline phosphatase activity is greater in extrahepatic cholestasis, while the albumin level is lower [2797]. Variations of the bilirubin level and parallel increase of bilirubin and phosphatase speak for extrahepatic cholestasis. Liver biopsy is also of limited help and only after several weeks of jaundice. Extrahepatic cholestasis involves both parenchyma and interlobular bile ducts. Intrahepatic cholestasis involves only the parenchyma, including the ductules, and stops at the periportal ductules. Demonstration of cholestasis beyond this point proves extrahepatic cholestasis. The characteristics of extrahepatic cholestasis may not appear until after several weeks of complete obstruction. These characteristics are bile plugs in interlobular bile ducts in the portal tracts, bile extravasates around these ducts, and bile infarcts. Since these features do not necessarily and do not uniformly develop, their absence does not exclude extrahepatic obstruction. Proliferation of bile ducts rather than ductules suggests extrahepatic cholestasis. Ductular proliferation, inflammatory reaction including infiltration with eosinophils, and periportal membrane formation are more conspicuous in intrahepatic cholestasis, except for instances of extrahepatic cholestasis with severe secondary infection. In extrahepatic cholestasis the hepatic-cell plates are often very thin. The optimal time for liver biopsy is 3 weeks after the onset of jaundice in such cases. It should be repeated 2 weeks later

if the initial biopsy findings are inconclusive. After this time biopsy becomes dangerous (see Dangers, under Indications, Contraindications, and Dangers, Chap. 39). Frequently exploratory laparotomy is the only reliable means for differentiation. Narrow common and hepatic bile ducts in a patient with laboratory signs of cholestasis are strong evidence for intrahepatic cholestasis. The only exception is a carcinoma at the junction of the hepatic ducts at the hilus of the liver obstructing both main branches.

Recognition of a Space-occupying Lesion

In the presence of hepatic neoplasms, abscesses, and amebic "abscesses," alkaline phosphatase activity and Bromsulphalein retention are often increased even in the absence of jaundice. In carcinoma metastases, the serum-gamma globulin level is usually normal, while in the other conditions it is elevated. The flocculation test results are usually normal, which assists in the differential diagnosis from cirrhosis. However, where there is incomplete biliary obstruction with little or no jaundice, occasionally alkaline phosphatase activity may be greatly increased and Bromsulphalein retention moderately so. In the presence of jaundice, the differentiation of carcinoma metastases from extrahepatic biliary obstruction may become a serious problem. Reduction of urinary urobilinogen excretion speaks for obstruction. The results of liver biopsy depend upon the chance of reaching the lesion with the needle.

Differential Diagnosis between Acute Hepatitis and Cirrhosis

Most tests for hepatic-cell degeneration as well as for cholestasis are equally abnormal in cirrhosis or hepatitis. The mesenchymal reaction is more extensive in cirrhosis than in hepatitis, and therefore serum gamma globulin and zinc sulfate turbidity are much higher in cirrhosis. The thymol turbidity tends to be less abnormal than the cephalin flocculation in nutritional cirrhosis but not necessarily so in postnecrotic cirrhosis. A lowered serum-albumin level and reversed A/G ratio speak for cirrhosis. However, the best laboratory procedure for the differentiation of hepatitis and cirrhosis is liver biopsy. The history alone is often helpful in the differential diagnosis. In addition, nodularity and blunting of the liver edge, enlargement of the spleen, esophageal varices, spider nevi, and anemia favor the diagnosis of cirrhosis. Never-

theless, laboratory information, particularly liver biopsy, often establishes the presence of cirrhosis in patients in whom the clinical picture suggests acute hepatitis.

Transition of hepatitis into cirrhosis is suggested by persistent elevation of the gamma globulin level at a time when the results of other hepatic tests have returned to normal.

Differentiation of Cirrhosis from Carcinoma

PRIMARY CARCINOMA VS. CIRRHOSIS. Primary carcinoma in the presence of cirrhosis or independent of it is suggested by very high levels of serum-alkaline phosphatase activity or by a rise of the serum mucoproteins. Demonstration of carcinoma in biopsy specimens is diagnostic; its absence does not exclude carcinoma. Tenderness and unexplained fever with increasing hepatic enlargement in a known cirrhotic patient speak for the development of a primary carcinoma.

SECONDARY CARCINOMA VS. CIRRHOSIS. The differentiation between metastatic carcinoma and cirrhosis or fatty liver is difficult. Secondary carcinoma is associated with increased serum-alkaline phosphatase activity and Bromsulphalein retention, while other signs of cholestasis may be absent. The gamma globulin level is frequently normal, as may be the other serum-protein reactions. The mucoproteins are high, as in primary carcinoma. The findings of carcinoma metastases in the liver biopsy are unequivocal. The presence of a coarse nodular liver with a sharp edge is suggestive of a metastatic lesion.

Differentiation between Benign and Malignant Biliary Obstruction

Permanent absence of urobilinogen from the urine more frequently occurs in malignant obstruction. An often quoted and less commonly observed exception is the temporary relief afforded by sloughing of necrotic tissue from a tumor of the papilla of Vater. In benign obstruction by strictures or stones, urinary urobilinogen excretion fluctuates and often is increased. The fluctuating urobilinogen excretion in calculous obstruction is produced by ball-valve action of the stone. Hepatic-cell degeneration is usually more apparent in malignant obstruction than in calculous obstruction and is best reflected in a low cholesterol/ester ratio. This also is seen in protracted benign obstruction or in secondary purulent infection in benign obstruction, particularly in strictures.

Microscopic examination of aspirated duodenal contents has limited value. The presence of cholesterol or bilirubin crystals speaks for stones; leukocytes speak for a purulent process. Exfoliated tumor cells are diagnostic for malignancy. Radiologic demonstration of stones or duodenal lesions is helpful. Demonstration of blood in the stools or weight loss is of little value, since it occurs in benign or malignant obstruction. The history and physical examination offer relatively little help except in the case of strictures. Colicky pains or chills with fever suggest stones; masses or an enlarged gallbladder indicate malignant obstruction (Courvoisier's law).

Appraisal of the Degree of Hepatic Insufficiency

The problem of appraisal of the degree of hepatic insufficiency occurs primarily in the management of the jaundiced patient after the diagnosis has been established. For this, serial laboratory tests are of the greatest help. Few of the tests lend themselves to quantitative comparison. The best are the cholesterol/ester ratio and the serum cholinesterase, which are equally useful in surgical and medical jaundice. In hepatitis, the serum-bilirubin level often suffices. Serum-albumin levels serve this purpose in cirrhosis. The results of the flocculation tests are of limited value. In milder degrees of hepatic insufficiency, variations in Bromsulphalein retention are helpful. Liver biopsy, especially as an indication of the efficiency of treatment, has been widely used. In cirrhosis, caution in interpretation is necessary, because the biopsy specimen may not be representative of the liver as a whole.

Indication of Severe Hepatic Failure (Alarm Signals). The following laboratory indications, listed in order of importance, are the most helpful in imminent hepatic failure: prothrombin concentration less than 20 per cent, with no response to vitamin K; precipitous drop in total serum cholesterol, cholesterol/ester ratio, and serum cholinesterase; rapid rise of serum bilirubin; aminoaciduria with positive Millon reaction for tyrosinuria; elevation of nonprotein nitrogen.

Clinically, hemorrhagic tendencies, fetor hepaticus, central nervous system manifestations with coma, rapid increase of jaundice, and rapid decrease of liver size are the most important signs.

Demonstration of Hepatic Injury in Nonjaundiced Patients (Screening Tests). The problem of demonstrating hepatic injury in nonjaundiced pa-

tients occurs primarily in screening for anicteric or preicteric viral hepatitis during an epidemic, for toxic damage after exposure to poisons, mainly industrial in nature, and for injury resulting from the administration of drugs for clinical or experimental purposes. Since otherwise healthy persons are examined, minor alterations of hepatic function are significant. False positive results and abnormal results in carriers of hepatitis sometimes interfere with the interpretation; isolated observations are unconvincing.

Determination of the following is recommended: urinary bilirubin and urobilinogen excretion, Bromsulphalein retention, prompt-reacting serum bilirubin, cephalin flocculation, and thymol turbidity. For the testing of drugs, hippuric acid synthesis and galactose clearance can be added.

Differential Diagnosis of Hepatomegaly in the Nonjaundiced Patient

The laboratory tests are of limited value in the differential diagnosis of hepatomegaly. Results may be normal or abnormal in almost any condition responsible for hepatic enlargement, including cirrhosis, fatty liver, carcinoma metastases, passive congestion, and amyloidosis. The determination of Bromsulphalein retention, cephalin flocculation, thymol turbidity, gamma globulin, alkaline phosphatase activity, urinary urobilinogen, and cholesterol/ester ratio are recommended for this purpose. Abnormal results of all speak for nonnutritional cirrhosis, while normal thymol turbidity with all the other results abnormal suggests nutritional cirrhosis. The incidence and degree of abnormal Bromsulphalein retention, gamma globulin elevation, cephalin flocculation, and urobilinogenuria are greatest in cirrhosis, as are the serum-albumin and cholinesterase levels, if malnutrition is excluded. Abnormal results in hippuric acid and galactose-tolerance tests are also more frequently found in cirrhosis. Cholesterol ester reduction, if present, strongly suggests cirrhosis. Low serum-mucoprotein values indicate postnecrotic cirrhosis or hepatitis [1274]. Normal results in all the tests almost exclude cirrhosis. Increased serum-alkaline phosphatase activity points to primary or secondary carcinoma (see Chap. 58), especially if associated with increased Bromsulphalein retention. In congestive failure the Bromsulphalein retention is out of proportion to the abnormalities in the other tests. In granulomas, serum-gamma globulin increase exceeds changes in the other tests. In fatty

livers no characteristic patterns are found except for frequently increased Bromsulphalein retention. These tendencies may be helpful but seldom are crucial in the differential diagnosis.

Liver biopsy is often diagnostic without reservation and therefore the method of choice for the differential diagnosis of hepatomegaly. The incidence of positive findings is increased by gross inspection and by serial section of the biopsy specimen for tumor nodules and granulomas. The histologic appearance away from the lesions is not diagnostic in carcinoma metastases, less frequently diagnostic in granulomatous diseases, and only exceptionally diagnostic in cirrhosis. Other laboratory findings, including the blood count, congo red retention for amyloidosis, and bacteriologic and serologic studies in granulomas, assist in the differential diagnosis.

Differential Diagnosis of Hepatomegaly in Children

The causes of hepatomegaly in children are the same as in adults, including infections, cardiac failure, hepatitis, cirrhosis, blood dyscrasias, neoplasms, especially neuroblastomas and primary hepatic carcinomas, metabolic disorders and anomalies. Glycogen-storage disease without splenomegaly and lipid-storage disease with splenomegaly are the important metabolic diseases. In addition, fatty metamorphosis on a nutritional basis develops in children far more rapidly than in adults, for instance following acute enteritis or diarrhea. Liver biopsy, even in young infants, may be of greater value in the differential diagnosis than laboratory tests [1172, 1713, 2268].

Tests of Value in Surgical Management

Newer developments in medical and surgical management, especially the introduction of antimicrobial and chemotherapeutic agents as well as electrolyte and blood-replacement therapy, have reduced contraindications to surgery to the point where very few absolute contraindications exist based on laboratory findings. Relative contraindications, requiring improvement of hepatic function if possible before surgery, are a prolongation of the prothrombin time, reduced cholesterol/ester ratio, azotemia, and aminoaciduria. The finding of metastatic carcinoma in a needle biopsy specimen usually contraindicates definitive surgery.

Successful relief of extrahepatic biliary obstruction is indicated by appearance of urobilinogen in the urine and reduction in serum bilirubin.

Serum-alkaline phosphatase activity does not return to normal so rapidly as the serum bilirubin.

General Uses of Hepatic Tests

Minimal Number of Determinations for Routine Study

1. Cephalin flocculation
2. Thymol turbidity
3. Zinc sulfate turbidity
4. Gamma globulin turbidity
5. Urinary urobilinogen excretion
6. Urinary bilirubin excretion
7. Bromsulphalein retention
8. Serum-bilirubin level
9. Serum-alkaline phosphatase activity
10. Total serum cholesterol and cholesterol esters
11. Serum albumin

Tests in Infancy and Childhood. The same hepatic tests can be used in the younger age groups as in adults. The tolerance tests require smaller doses. For instance, in the oral galactose-tolerance test 0.5 gm per kg body weight of galactose is given. The hippuric acid test is also modified on a weight basis [2050]. The total serum proteins are low at birth and rise with increasing maturity [2708, 3359]. In infancy gamma globulin is low, and the globulin fraction rises more rapidly than albumin. The results of the turbidity tests are not reliable in infancy [1143]. They may be normal even with severe hepatic injury. Levulose tolerance, Bromsulphalein retention, cephalin flocculation, and thymol turbidity are abnormal in the first week of life [776, 2873]. In the neonatal period, icterus neonatorum influences bile pigment metabolism [3528]. Normally the serum-bilirubin level reaches a peak on the second day of life, but if erythroblastosis is present it continues to rise until the fourth day [1563]. Bilirubin tolerance is not altered [2007], but the fecal urobilinogen is relatively low in infants, because bacterial growth in the colon has not reached its full development [3298]. The serum-alkaline phosphatase activity is higher in children than in adults and rises even higher in hepatic diseases [2709]. The prothrombin time is prolonged at birth and on the second day of life, particularly if the mother has not received vitamin K [1700]. Liver biopsy can be performed even in the neonatal period [428].

Tests in Animal Experiments. **LARGE ANIMALS.** In monkeys, dogs, cats, and rabbits, the same tests can be used as in man, since blood and urine samples can easily be collected. The cephalin floccula-

tion is normally very strongly positive in healthy animals of every species [1375] except the monkey. Serum bilirubin, thymol turbidity, cephalin flocculation, and alkaline phosphatase activity have been used in monkeys [957]. In dogs the results of the various tests have been compared with morphologic changes [1234]. In dogs, cats, and rabbits for detection of hepatic damage produced by drugs, diets, or operative procedures on the biliary tract, the dye tests are the most useful; Bromsulphalein retention 15 minutes after administration of 10 mg per kg [1234] gives reliable results [382, 1606, 1649, 2142, 2303, 2484, 3270, 3508]. Rose bengal clearance is almost as reliable [3270]. The serum-alkaline phosphatase activity is increased in biliary obstruction and to a lesser degree in hepatic-cell degeneration without obstruction [835, 838, 1090, 1551, 3270]. The prothrombin time is slightly less sensitive [838, 1234, 3270]. Determinations of serum protein [1234, 1919, 3270] and the cholesterol/ester ratio [3320] are useful, while thymol turbidity [400, 1234] and zinc sulfate turbidity [1234] are of less value. The

intravenous galactose tolerance [838, 1234], bilirubin tolerance [2303, 3270], icterus index or serum bilirubin [1234], and urinary urobilinogen [1234, 3270] are apparently of little value.

SMALL ANIMALS. The simplest method to determine liver damage in mice is the demonstration of bilirubin in the urine [1523]. Adaptations of the Bromsulphalein clearance [510, 1820, 1824, 2990] and serum-bilirubin determinations [510, 1820, 1824] are helpful. Hepatic-cell damage is best recognized in rats by a simultaneous increase of alkaline phosphatase activity [1820, 2492, 3537] and reduction of esterase in serum and also in the liver [1820, 1821]. The esterase reduction does not occur in acute injury [2637]. Reduction of xanthine oxidase and increase of transaminase activity in serum also provide a valuable index of hepatic injury in rats. Total protein determinations, including albumin/globulin partition, have been used [253], as have adaptations of the hippuric acid test [103], but species differences create difficulties [2331]. The flocculation tests, including thymol turbidity, are of little value [510].

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ADDENDUM

Since the completion of the manuscript many pertinent papers have appeared or have come to the attention of the authors. In this Addendum brief reference is made to reports which appear to be of special interest. This introduces a strong subjective element, and many important items may be omitted.

Chapter 4

The site of action of insulin has been more specifically defined. In addition to its role in glycolysis (see Chap. 62), insulin is necessary for the condensation of acetyl coenzyme A and oxalacetate, the point of entrance to the Krebs cycle and the crossroad of intermediary metabolism of carbohydrate, fat, and protein (Beatty, C. H., and West, E. S.: *J.Biol.Chem.* 215:661, 1955).

Chapter 6

Paper electrophoresis (see Chap. 32) has provided some information as to the serum-protein-bound lipids and carbohydrates in liver disease. Alpha lipoproteins, which are reduced with elevated serum-lipid concentrations [1883], disappear regularly at the height of extrahepatic biliary obstruction and occasionally in hepatitis and cirrhosis. Abnormal beta lipoproteins have been found in primary biliary cirrhosis (Russ, E. M., Raymunt, J., and Barr, D. P.: *J.Clin.Investigation* 35:133, 1956), and their fractionation by ultracentrifugation is altered in hepatitis (Pierce, F. T., Jr., Kimmel, J. R., and Burns, T. W.: *Metabolism* 3:228, 1954).

The protein-bound carbohydrates as a whole rise in most liver diseases. The increase in the fraction bound to gamma globulin does not necessarily parallel the protein elevation. This might provide a means of characterizing the gamma

globulin increment (reaction globulin) (see Globulin Formation, Chap. 14) by its carbohydrate content.

Chapter 7

The interest in the intermediary metabolism of carbohydrates in liver disease has stimulated the study of some of the associated enzymes especially for diagnostic purposes (see also Chap. 35 in this Addendum). Aldolase is the enzyme which splits fructose diphosphate into two triose phosphates during anaerobic glycolysis. The activity of this enzyme in serum is increased in experimental liver damage produced by carbon tetrachloride (Sibley, J. A., Higgins, G. M., and Fleisher, G. A.: *A.M.A.Arch.Path.* 59:712, 1955) and in patients with acute hepatitis (Løken, F.: *Scandinav.J.Clin. & Lab.Investigation* 8:175, 1956). In animals and patients with extrahepatic biliary obstruction and in chronic liver disease, normal or only moderately increased activity is found.

Chapter 8

The method of partition of vitamin A ester and alcohol in liver and serum has been placed on a sounder basis. The plasma level of vitamin A alcohol is said to be controlled by a mechanism different from the tissue concentration and dependent upon specific carrier proteins in plasma (High, E. G., and Wilson, S. S.: *Arch.Biochem.* 62:163, 1956).

The relation between serum iron and hepatic iron has been somewhat elucidated by the observation that the release of ferrous iron from ferritin, which contains mainly ferric iron, depends on the availability of sulfhydryl groups which release the ferrous iron and protect it from oxidation. In liver hypoxia, plasma iron increases (Mazur, A., Baez, S., and Shorr, E.: *J.Biol.Chem.* 213:147,

1955). Blockage of the sulfhydryl groups stimulates the accumulation of iron in liver slices (Saltman, P., Fiskin, R. D., Bellinger, S. B., and Alex, T.: *J.Biol.Chem.* **220**:751, 1956). The passage of iron through the cell membrane itself does not depend on active metabolic processes.

Chapter 9

The conjugation of cholic acid with taurine and glycine takes place in the microsomal fraction of the hepatic cell with the help of ATP and coenzyme A as in detoxification reactions (Elliott, W. H.: *Biochem.J.* **62**:427, 1956).

Chapter 11

Bilirubin appears to be bound to albumin in serum regardless of the type of its diazo reaction, and therefore differences in the van den Bergh reaction are not related to protein binding (Klat-skin, G., and Bungards, L.: *J.Clin.Investigation* **35**:537, 1956). Two independent groups of workers have just clarified the principle of the bilirubin partition according to van den Bergh. Indirect-reacting bilirubin is not esterified and is poorly soluble in water, so that its urinary excretion is prevented. It is coupled with diazo reagent only with the help of additional substances such as alcohol. In contrast, direct-reacting bilirubin is a diglucuronic acid ester which is readily soluble in water. It can be transformed to free bilirubin by beta glucuronidase (Schmid, R.: *Science* **124**:76, 1956; Billing, B. H., and Lathe, G. H.: *Biochem.J.* **63**:6P, 1956).

Crystallization and determination of the formulas of *d*-urobilin and *d*-urobilinogen have been accomplished (Watson, C. J., and Lowry, P. T.: *J.Biol.Chem.* **218**:633, 1956; Lowry, P. T., Cardinal, R., Collins, S., and Watson, C. J.: *J. Biol. Chem.* **218**:641, 1956).

Chapter 12

Bile seems to play a minor role in the absorption of fat in comparison to pancreatic juice (Wells, M. H., Shingleton, W. W., and Sanders, A. P.: *Proc.Soc.Exper.Biol.& Med.* **90**:717, 1955).

Chapter 13

With improvement in tissue culture techniques, massive cultures of hepatic cells have been obtained. Some of the cells in such cultures assume a spindle-cell or round-cell shape, although they contain much glycogen, in contrast to fibroblasts in cultures (Westfall, B. B., Evans, V. J., Shan-

non, J. E., Jr., and Earle, W. R.: *J.Nat.Cancer Inst.* **14**:655, 1953-1954). Nevertheless, the transformation of hepatic epithelial cells into mesenchymal elements previously suggested [1083] is still not fully established. Normal human hepatic epithelial cells have been kept in serial cultures for many passages by roller-tube techniques (Chang, R. S.: *Proc.Soc.Exper.Biol.& Med.* **87**:440, 1954) and have been shown to develop the characteristics of an undifferentiated neoplasm (Moore, A. E., Southam, C. M., and Sternberg, S. S.: *Science* **124**:127, 1956).

Chapter 14

Phagocytosis of colloidal material by Kupffer cells has been studied more thoroughly. The engulfed material first appears free in the cytoplasm and then becomes surrounded by a covering of submicroscopic particles, resembling a vesicle (Parks, H. F.: *Anat.Rec.* **125**:1, 1956).

Chapter 18

The rate of disappearance of colloidal radioactive gold from the circulation has been used for the estimation of total hepatic blood flow (Vetter, H., Falkner, R., and Naumayr, A.: *J.Clin.Investigation* **33**:1594, 1954). This is also supported by perfusion studies using colloidal chromic phosphate (Brauer, R. W., Leong, G. F., McElroy, R. F., and Holloway, R. J.: *Am.J.Physiol.* **184**:593, 1956). The gold method appears simpler than the determination of the extraction of Bromsulphalein for which hepatic catheterization is necessary. In cirrhosis the rate of disappearance of colloidal gold is greatly reduced. Intravenous injection of ethanol increases hepatic blood flow supposedly by lowering the resistance in the splanchnic bed (Mendeloff, A. I.: *J.Clin.Investigation* **33**:1298, 1954).

Evidence has been presented against the dependence of hepatic regeneration on the portal blood supply (Weinbren, K.: *Brit.J.Exper.Path.* **36**:583, 1955). Additional evidence against the streamlines of flow in the portal vein has been brought forward with the help of radioiodine-tagged rose bengal (Cole, J. W., Krohmer, J., Bonte, F. J., and Schatten, W.: *Surg.,Gynec.& Obst.* **102**:543, 1956). However, predominance of hepatic disease in the left lobe in infancy has been correlated with alteration of hepatic blood flow caused by reduced circulation in the left lobe in the neonatal period (Emery, J. L.: *J.Path.& Bact.* **69**:219, 1955).

The regulation of the blood flow within the sinusoids has been observed with vital microscopy. The blood flow can be altered by increased size of the Kupffer cells and hepatic cells, by local congestion, or by movement of entire hepatic-cell plates (Peters, R.: *Acta Hepatologica* 4:28, 1956).

Chapter 20

To the agonal and postmortal changes should be added the better staining of cytoplasmic pentose nucleic acids with pyronin in autopsy specimens (Himes, M. B., Rizski, R., Hoffman, J., Pollister, A. W., and Post, J.: *A.M.A.Arch.Path.* 58:345, 1954).

Chapter 21

The opinion is gaining ground that in extrahepatic biliary obstruction, mechanical factors are of minor importance for the development of jaundice and even for the dilatation of the ducts [1171]. Several papers concerning chronic idiopathic jaundice with unidentified pigment in the hepatic cells (Dubin-Johnson syndrome) have appeared (Sprinz, H., and Nelson, R. S.: *Ann.Int. Med.* 41:952, 1954; John, G. G., and Knudtson, K. P.: *Am.J.Med.* 21:138, 1956; Nelson, R. S.: *Gastroenterology* 30:301, 1956). The pigment gives fat reactions. The condition must be differentiated from familial nonhemolytic icterus (Baroody, W. G., and Shugart, R. T.: *Am.J.Med.* 20:314, 1956) and posthepatic jaundice [1576]. The jaundice seems to be aggravated by intercurrent diseases or alcoholism and may be associated with such clinical symptoms as lassitude, slight enlargement of the liver, which is tender, and vague upper abdominal pain (Brown, N. L., and Schnitka, T. K.: *Am.J.Med.* 21:292, 1956). The condition has been reported in siblings.

Chapter 23

Hepatic necrosis may coincide with release of polysaccharides into the blood stream (Martirani, I., Wajchenberg, B. L., Hoxter, G., and Cintra, A. B. U.: *Gastroenterology* 30:286, 1956).

Ketogenesis is greatly decreased in experimental and human hepatic injury. This is associated with decreased butyrate and pyruvate oxidation by mitochondria and has been explained by a specific defect in acetate activation (Recant, L.: *J.Lab.& Clin.Med.* 48:165, 1956; Fischer, G. L., and Recant, L.: *J.Lab.& Clin.Med.* 48:171, 1956).

The rise of sulfhydryl groups in the serum in

hepatic coma (Walshe, J. M., and Senior, B.: *J.Clin.Investigation* 34:302, 1955) may be related to the formation of methylmercaptan, the substance responsible for fetor hepaticus but not for the hepatic coma. Fetor following choline administration may be confused with fetor hepaticus but is rather the result of formation of trimethylamine (Söderström, N.: *Acta med.Scandinav.* 153:443, 1956).

In hepatic coma, respiratory alkalosis parallels an elevated blood-ammonia level (Vanamee, P., Poppell, J. W., Glicksman, A. S., Randall, H. T., and Roberts, K. E.: *A.M.A.Arch.Int.Med.* 97:762, 1956).

The relation of blood ammonia to hepatic coma remains an intriguing problem, although some progress has been made (Bessman, S. P.: *Ann.Int. Med.* 44:1037, 1956). The correlation between coma and elevated ammonia levels has been confirmed (Zimmerman, H. J., and Korn, R. J.: *Am.J.M.Sc.* 231:177, 1956). The effect of antibiotics in inhibiting intestinal ammonia formation remains unsettled. However, the neurologic symptoms which develop in some patients with cirrhosis following oral methionine administration were inhibited by antibiotics (Phear, E. A., Ruebner, B., Sherlock, S., and Summerskill, W. H. J.: *Clin.Sc.* 15:93, 1956). The therapeutic use of carbonic anhydrase inhibitors for diuretic purposes in cirrhosis may precipitate hepatic coma (Webster, L. T., and Davidson, C. S.: *Proc.Soc.Exper.Biol.& Med.* 91:27, 1956).

Chapter 26

Further evidence for the functional integrity of the fatty liver is provided by the observation that regeneration after partial hepatectomy is not impaired in severe nutritional fatty metamorphosis (Sutherland, A. M.: *J.Path.& Bact.* 71:403, 1956).

Chapter 28

In coarse nodular or postnecrotic cirrhosis, new formation of lobules within the nodules has been demonstrated as a result of undisturbed repair after massive injury, while, in contrast, in portal or septal cirrhosis, persistence of the injury leads to small nodules (Smetana, H. F.: *Lab.Investigation* 5:175, 1956).

The vascular anastomoses in cirrhosis possibly account for the bacteremia with Gram-negative bacilli developing in severe liver disease (Martin, W. J., Spittel, J. A., Morlock, C. G., and Baggenstoss, A. H.: *A.M.A.Arch.Int.Med.* 98:8, 1956).

Chapter 29

Elevation of venous pressure in catheters wedged in hepatic veins in the absence of elevated peripheral venous pressure is considered diagnostic of cirrhosis (Taylor, W. J., and Myers, J. D.: *Circulation* 13:368, 1956).

Additional support for surgical shunt in portal hypertension is forthcoming on the basis of observations on increasing clinical material (Ellis, D. S., Linton, R. R., and Jones, C. M.: *New England J.Med.* 254:931, 1956). With Bromsulphalein retention below 10 per cent, the chances of postoperative complications are negligible, whereas a serum-albumin level below 3.0 gm favors a high incidence of such complications (Ebeling, W. C., Bunker, J. P., Ellis, D. S., French, A. B., Linton, R. R., and Jones, C. M.: *New England J.Med.* 254:141, 1956). The seriousness of hemorrhage from esophageal varices is indicated by the fact that over three-fourths of the patients die within a year of their first massive hemorrhage. One cause of coma and death following hemorrhage is absorption of ammonia formed by breakdown of the blood in the intestinal tract. This can be reduced by administration of laxatives and enemas (Welch, C. S., Kiley, J. E., Reeve, T. S., Goodrich, E. O., and Welch, H. F.: *New England J.Med.* 254:493, 1956). Emergency treatment of bleeding varices by esophageal tamponade followed by transthoracoesophageal suture of the varices has decreased the mortality rate (Linton, R. R., and Ellis, D. S.: *J.A.M.A.* 160:1017, 1956).

Chapter 30

Biliary concretions and inflammatory lesions of the biliary tract have been produced in rabbits by feeding dihydrocholesterol, indicating dietary production of gallstones and the irritating effect of derivatives of biliary substances (Bevans, M., and Mosbach, E. H.: *A.M.A.Arch.Path.* 62:112, 1956).

Chapter 35

The activity of serum glutamic oxalacetic aminophosphatase (or transaminase) increases before other clinical or laboratory signs become apparent in preicteric stages of viral hepatitis and of toxic hepatic injury. This increase is a sensitive indicator of hepatic-cell destruction (Wróblewski, F., and LaDue, J. S.: *Ann.Int.Med.* 43:345, 1955; *J.A.M.A.* 160:1130, 1956). This test is now widely used as a screening procedure. Transaminase also rises in the presence of metastatic tumor in the

liver; this permits differentiation from osseous metastases, in the presence of which the serum-alkaline phosphatase level is elevated but not the transaminase level (Wróblewski, F., and LaDue, J. S.: *Cancer* 8:1155, 1955). The test can also be used in laboratory animals, and increased activity in serum has been found in mice with experimental viral hepatitis (Friend, C., Wróblewski, F., and LaDue, J. S.: *J.Exper.Med.* 102:699, 1955).

Chapter 37

The correction of Bromsulphalein retention to take into account the effect of bilirubinemia has not increased the practical advantage of this test over others in the differential diagnosis of jaundice (Metzler, C., Hoffbauer, F. W., and Benson, E.: *J.Lab.& Clin.Med.* 47:519, 1956).

The use of serum-iron levels in the differential diagnosis of jaundice has been extended, and the late appearance of high levels in viral hepatitis may indicate a protracted course (Stone, C. M., Jr., Rumball, J. M., and Hassett, C. P.: *Ann.Int.Med.* 43:229, 1955).

Chapter 38

Percutaneous transhepatic cholangiography, with injection of 20 to 60 ml Diodrast through a long 18-gauge needle into an intrahepatic bile duct, has resulted in demonstration of the bile ducts in almost 80 per cent of cases (Remolar, J., Katz, S., Rybak, B., and Pellizari, O.: *Gastroenterology* 31:39, 1956). If the technique proves safe, it may be a great diagnostic advance.

Splenoportography has been used to demonstrate circulatory blocks in infrahepatic portal hypertension (Rodriguez, H. F., Bonnet, R. D., and Rodriguez-Perez, D.: *Ann.Int.Med.* 44:773, 1956).

Chapter 41

Interference with the metabolism of the hepatic cells is more important than disturbances of circulation in various forms of toxic hepatic necrosis as indicated by measurement of hepatic temperature and blood flow (Stoner, H. B.: *Brit.J.Exper.Path.* 37:176, 1956). Another piece of evidence for the concept that circulatory disturbances are the result rather than the cause of hepatocellular damage has been provided by studies with intravenous injection of beryllium sulfate solutions, producing peripheral necrosis (Cheng, K. K.: *J.Path.& Bact.* 71:265, 1956).

Changes in hepatic-cell nuclei persisting after histologic repair following a single toxic injury

have been held responsible for the more violent response to a second injury (Hoffman, J., Himes, M. B., Klein, A., Poulos, V., and Post, J.: *A.M.A. Arch.Path.* 62:96, 1956).

Two instances of hepatocellular injury following carbarsone administration have been reported (Nelson, R. S.: *J.A.M.A.* 160:764, 1956).

Among the antiarthritic drugs, phenylbutazone has been found occasionally to produce hepatocellular injury (Mauer, E. F.: *New England J. Med.* 253:404, 1955). The number of instances of drug-induced primary cholestasis in the form of allergic cholangiolitis is increasing. Sulfadiazine has been accused of causing it (Hoffman, F. G.: *Gastroenterology* 29:247, 1955), as has para-aminobenzyl caffeine hydrochloride (Borges, F. J., Revell, S. T. R., Jr., and O'Malley, W. E.: *J.Lab.& Clin.Med.* 47:735, 1956).

Many instances of chlorpromazine-induced cholangiolitis have been reported (Van Ommen, R. A., and Brown, C. H.: *J.A.M.A.* 157:321, 1955; Redeker, A. G., and Balfour, D. C., Jr.: *Gastroenterology* 29:882, 1955; McHardy, G., McHardy, R., and Canale, S.: *Gastroenterology* 29:184, 1955; Movitt, E. R., Meyer, M. C., Snell, A. M., Goldman, M. J., Gibson, J. R., Sullivan, B. H., Jr., Webster, J. G., and Stone, R. B.: *Gastroenterology* 28:901, 1955; Gold, H., Rosenberg, F., and Campbell, W.: *Ann.Int.Med.* 43:745, 1955; Stern, A. A., and Wright, A. W.: *J.A.M.A.* 161:508, 1956). The incidence of jaundice following chlorpromazine administration seems much higher than after other drugs which induce primary cholestasis. Jaundice develops in about 2 per cent of all patients receiving the drug. Moreover, biopsies in nonjaundiced patients receiving chlorpromazine revealed histologic changes similar to those in patients with jaundice (Sims, J. L., Bremer, E. M., and Huston, E. S.: *J.Lab.& Clin. Med.* 46:952, 1956). The jaundice occasionally lasts for many months, and a few fatalities have occurred. At autopsy, intrahepatic cholestasis with small areas of central necrosis have been seen, which are probably only a nonspecific shock effect. Some of the patients died following surgical exploration of the biliary tract, possibly because they were more susceptible to stress. The extrahepatic bile ducts were not dilated. Whether the increase in resistance of the choledochoduodenal sphincter demonstrated in dogs (Menguy, R. B., Grindlay, J. H., and Cain, J. G.: *Gastroenterology* 30:752, 1956) exists in man is questionable. Occasionally the primary cholestasis is

associated with hepatocellular injury reflected in histologic changes and abnormal results of the flocculation tests. In rats with ethionine-induced hepatocellular injury, chlorpromazine produces severe jaundice.

A type of nonspecific reactive hepatitis with focal hepatocellular damage, eosinophilia, and abnormal results of flocculation tests occurs in Q fever (Gerstl, B., Movitt, E. R., and Skahen, J. R.: *Gastroenterology* 30:813, 1956).

Chapter 42

Serum of patients with presumed viral hepatitis has been found to have cytotoxic effects in tissue cultures, like serums in other virus diseases (Rightsel, W. A., Keltsch, R. A., Tekushan, F. M., and McLean, I. W., Jr.: *Science* 124:226, 1956).

Three self-limited attacks of viral hepatitis, each a year apart, have been described in a drug addict. They have been explained either by the presence of different viruses or by a single virus incapable of eliciting an antibody reaction (Havens, W. P., Jr.: *Ann.Int.Med.* 44:199, 1956).

Chapter 43

Encephalitis has been observed clinically in acute viral hepatitis (McMath, W. F. T.: *Brit. M.J.* 1:270, 1955). In fatal cases, myocarditis with necrosis of isolated fibers and diffuse serous exudation has been seen and is associated with electrocardiographic changes during life (Saphir, O., Amromin, G. D., and Yokoo, H.: *Am.J.M.Sc.* 231:168, 1956).

Chapter 44

The search for residual changes following viral hepatitis continues. In veterans who had hepatitis during their military service, the incidence of borderline hepatic abnormalities exceeds that in veterans without a history of viral hepatitis. However, some abnormalities have also been found in a significant number of the latter group. Other etiologic factors have to be considered. Moreover, with a symptom-free interval of more than 3 years between attacks of viral hepatitis, a new infection has to be considered (Neeffe, J. R., Gambescia, J. M., Kurtz, C. H., Smith, H. D., Beebe, G. W., Jablon, S., Reinhold, J. G., and Williams, S. C.: *Ann.Int.Med.* 43:1, 1955).

Chapter 46

An increasing number of instances of intrahepatic cholestasis have been described. They differ

pathologically from typical viral hepatitis. Nevertheless this etiology cannot be excluded (Johnson, H. C., Jr., and Doenges, J. P.: *Ann.Int.Med.* 44: 589, 1956; Lipschutz, E. W., and Capson, D.: *Ann.Int.Med.* 43:1037, 1955). ACTH therapy is beneficial and has been recommended as a therapeutic test for differentiation from biliary obstruction (Chalmers, T. C., Gill, R. J., Jernigan, T. P., Svec, F. A., Jordon, R. S., Waldstein, S. S., and Knowlton, M.: *Gastroenterology* 30:894, 1956). However, some instances of "cholangiolitic hepatitis" have been found morphologically hardly distinguishable from the usual form of viral hepatitis with single-cell necrosis and have appeared distinctly different from extrahepatic biliary obstruction (Gall, E. A., and Braunstein, H.: *Am.J. Clin.Path.* 25:1113, 1955).

The subacute hepatitis in adolescent girls has been described in more detail, especially in later cirrhotic stages. These stages are accompanied by arthritis, obscure febrile episodes, hormonal disturbances, and hypergammaglobulinemia. A relation to viral hepatitis is not clear (Bearn, A. G., Kunkel, H. G., and Slater, R. J.: *Am.J.Med.* 21:3, 1956).

Neonatal giant-cell hepatitis has been observed in siblings with possible blood-group incompatibility (Krainin, P., and Lapan, B.: *J.A.M.A.* 160: 937, 1956). In one instance, transition of giant-cell hepatitis into primary hepatic carcinoma was observed in a child who died before the age of three years (Roth, D., and Duncan, P. A.: *Cancer* 8:986, 1955).

Chapter 47

Secondary biliary cirrhosis is characterized by less hepatocellular regeneration, smaller and more irregular regenerative nodules, loose irregular septums, and more cholestasis than nutritional septal cirrhosis (Doehlert, C. A., Baggenstoss, A. H., and Cain, J. C.: *Am.J.Clin.Path.* 25:902, 1955).

Chapter 48

In congestive hepatic injury, severe jaundice has been reported in the absence of infarcts but frequently associated with rheumatic heart disease and auricular fibrillation (Parker, J. G., and Felder, L.: *Ann.Int.Med.* 43:1031, 1955). This has been connected with the degree of hepatic necrosis or fibrosis.

Chapter 50

Fatty liver and hepatocellular injury have been produced in rats who were fed human diets con-

sisting of spaghetti, olive oil, and wine (Ratnoff, O. D., and Patek, A. J., Jr.: *Proc.Soc.Exper.Biol. & Med.* 90:620, 1956). Various plant materials have produced portal fatty metamorphosis (Shils, M. E., Friedland, I., and Stewart, W. B.: *Proc. Soc.Exper.Biol.& Med.* 87:473, 1954). In fatty liver in general, the coenzyme A content of the liver decreases (Severi, C., and Fonnesu, A.: *Proc. Soc.Exper.Biol.& Med.* 91:368, 1956).

In the dietary necrosis associated with cystine and tocopherol deficiency, a metabolic lesion precedes histologic changes. It is characterized by failure of lipogenesis, of ketogenesis, and of oxidation of acetate to carbon dioxide (Chernick, S. S., Moe, J. G., Rodnan, G. P., and Schwarz, K.: *J.Biol.Chem.* 217:829, 1955; Rosecan, M., Rodnan, G. P., Chernick, S. S., and Schwarz, K.: *J.Biol.Chem.* 217:967, 1955).

Evidence has been presented that the necrosis-inhibiting effect of methionine is shared by cysteine but not by choline, and therefore the sulfhydryl rather than the methyl group is held responsible. This is supported by the observation that methionine is effective only if given before the intoxication which is supposed to interfere with its demethylation (Eger, W.: *Virchows Arch. f.path.Anat.* 328:536, 1956).

Chapter 51

Acute hepatic insufficiency in alcoholic persons with fatty livers has been correlated with the extent of cytoplasmic coagulation (see Mallory Bodies, under Cytoplasmic Degeneration, Chap. 22) (Phillips, G. B., and Davidson, C. S.: *A.M.A. Arch.Int.Med.* 94:585, 1954).

Chapter 52

A recent study on a large number of cases of cirrhosis in children points out the tendency to diagnose secondary biliary cirrhosis too readily, for instance, in viral hepatitis. Moreover, multinucleated giant cells have been encountered in almost all types of cirrhosis (Peace, R.: *A.M.A. Arch.Path.* 61:107, 1956).

Chapter 53

The problem of excess iron absorption and storage is still widely debated, especially in secondary hemochromatosis associated with anemia (Finch, S. C., and Finch, C. A.: *Medicine* 34: 381, 1955; Wyatt, J. P.: *A.M.A.Arch.Path.* 61:42, 56, 1956). The possibility has been suggested that

the intestinal area absorbing iron increases in secondary hemochromatosis.

An electrophoretically distinct protein fraction with great copper avidity has been found in hepatolenticular degeneration. The presence of this protein has been suggested as the basic cause of this disease (Uzman, L. L., Iber, F. L., Chalmers, T. C., and Knowlton, M.: *Am.J.M.Sc.* 231:511, 1956).

Chapter 54

Reference should be made to a very complete study on the role of liver biopsy in tuberculosis (Haex, A. J. C., and van Beek, C.: *Tuberculosis and Aspiration Liver Biopsy*, Haarlem, Holland, De Erven F. Bohn N. V., 1955).

Chapter 55

Granulomas caused by *Toxocara canis*, a nematode parasite in dogs which occasionally produces human visceral larva migrans, have been demonstrated in a liver biopsy specimen (Dent, J. H., Nichols, R. L., Beaver, P. C., Carrera, G. M., and Staggers, R. J.: *Am.J.Path.* 32:777, 1956).

Experimental infestation with *Fasciola hepatica* produces cirrhosis in rabbits which is not obstructive in nature but is caused by scarring of the migration tracts of the larvae, chronic cholangitis owing to the adult flukes in the bile ducts, hyperplasia of the portal tracts, and granulomas around the fluke eggs in the liver tissue (Urquhart, G. M.: *J.Path.& Bact.* 71:301, 1956).

Chapter 57

Extensive fibrosis of the liver associated with portal hypertension may result from coalescence of numerous microhamartomas (Meyenberg complexes). In one instance they have been associated with primary hepatic carcinoma (Parker, R. G. F.: *J.Path.& Bact.* 71:359, 1956).

Biopsy or aspiration of hemangiomas of the liver during operations may produce severe hemorrhage and should be performed only if the surgeon is ready to proceed with resection (Henson, S. W., Jr., Gray, H. K., and Dockerty, M. B.: *Surg.,Gynec.& Obst.* 103:327, 1956).

Chapter 59

Right or left hepatic lobectomies are being used with increasing frequency and some success for

the removal of primary or secondary neoplasms (Brunschwig, A.: *Cancer* 8:1226, 1956).

Chapter 60

Biologically false lupus erythematosus reactions (L. E. cells) have been found in cases of post-necrotic cirrhosis with high serum gamma globulin (Heller, P., Zimmerman, H. J., Rozengvaig, S., and Singer, K.: *New England J.Med.* 254:1160, 1956).

Chapter 61

In children with fibrocystic disease of the pancreas, postnecrotic cirrhosis occasionally occurs in the absence of any other etiologic factor (Montgomery, B. K., and Askanazy, C. I.: *Am.J.Clin. Path.* 26:630, 1956).

Chapter 62

Glucagon, like epinephrine and unlike insulin, decreases the conversion of glucose and fructose to fatty acids in liver slices and increases ketone body formation (Beaser, S. B.: *New England J. Med.* 255:173, 1956).

The role of the liver in inactivation of insulin has been studied further with the use of radio-iodine-tagged insulin (Williams, R. H.: *Metabolism* 5:128, 1956) and hepatic-cell fractions (Narahara, H. T., Tomizawa, H. H., Miller, R., and Williams, R. H.: *J.Biol.Chem.* 217:675, 1955). Injected insulin is rapidly and firmly bound in the hepatic cell. The enzyme system responsible for its degradation shows increased activity in the liver in diabetes mellitus, possibly because of inadequate insulinase inhibitors (Mirsky, I. A.: *Metabolism* 5:138, 1956; Gambassi, G., Maggi, V., Matarazzo, C.: *Acta med.Scandinav.* 153:201, 1956). Insulinase is not a very specific enzyme; it is proteolytic in nature and is found in the supernatant fluid of centrifuged hepatic cells.

Chapter 63

In liver disease, spontaneous arteriovenous shunts (apparently in the erythematous palms) are increased as suggested by the decreased arteriovenous oxygen difference (Silverstein, E.: *J.Lab.& Clin.Med.* 47:513, 1956).

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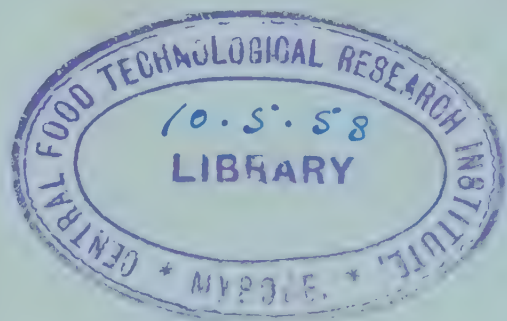
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